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VOLUME IV—1917

WITH THIRTY-ONE PLATES AND NINETY-FOUR TEXT FIGURES

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TABLE OF CONTENTS, VOLUME IV, 1917

No. 1, January

PAGE

The influence of certain climatic factors on the development of Endothia parasitica (Murr.) And. (with three text figures) Neil E. Stevens 1

Gunnera petaloidea Gaud., a remarkable plant of the Hawaiian Islands........................Vaughan MacCaughey 33

An interesting modification in Xanthium (with three text figures) Charles A. Shull 40

Endophyllum-like rusts of Porto Rico (with plates I–III) E. W. Olive and H. H. Whetzel 44

No. 2, February

Fifteen- and sixteen-chromosome Oenothera mutants (with nine text figures)..............................Anne M. Lutz 53

The influence of temperature on the growth of Endothia parasitica (with one text figure)................Neil E. Stevens 112

No. 3, March

Matroclinic inheritance in mutation crosses of Oenothera Reynoldsii (with four text figures) Carl D. La Rue and H. H. Bartlett 119

Duration of leaves in evergreens (with thirteen text figures) Vinnie A. Pease 145

The relation between evaporation and plant succession in a given area (with nine text figures)............Frank C. Gates 161

No. 4, April

The relation of some rusts to the physiology of their hosts (with plates IV and V)..........................E. B. Mains 179

The development of some species of Agarics (with plates VI–XI) A. W. Blizzard 221

The origin and development of the galls produced by two cedar rust fungi (with one text figure and plates XII–XVII)
J. L. WEIMER 241

NO. 5, MAY

The perennial scapose Drabas of North America
EDWIN BLAKE PAYSON 253
The osmotic concentration of the tissue fluids of Jamaican montane rain-forest vegetation
J. ARTHUR HARRIS AND JOHN V. LAWRENCE 268
The viability of radish seeds (Raphanus sativus L.) as affected by high temperatures and water content (with one text figure)
H. G. WAGGONER 299

NO. 6, JUNE

The taxonomy of the Agaricaceae......WILLIAM A. MURRILL 315
Observations on forest tree rusts (with two text figures)
JAMES R. WEIR AND ERNEST HUBERT 327
Endothia pigments I. (with six text figures)
LON A. HAWKINS AND NEIL E. STEVENS 336
Observations on an Achlya lacking sexual reproduction (with plate XVIII)..........................WM. H. WESTON 354
The rusts occurring on the genus Fritillaria (with three text figures)...........................CHARLES C. REES 368

NO. 7, JULY

Fertility in Cichorium intybus: the sporadic occurrence of self-fertile plants among the progeny of self-sterile plants (with two text figures)...........................A. B. STOUT 375
Inheritance of endosperm color in maize.....ORLAND E. WHITE 396
The influence of light and chlorophyll formation on the minimum toxic concentration of magnesium nitrate for the squash (with two text figures)..R. B. HARVEY AND R. H. TRUE 407
The use of the vibration galvanometer with a 60-cycle alternating current in the measurement of the conductivity of electrolytes (with one text figure)................NEWTON B. GREEN 411
Immunochemical studies of the plant proteins: proteins of the wheat seed and other cereals. Study IX..R. P. WODEHOUSE 417
The toxicity of galactose and mannose for green plants and the antagonistic action of other sugars toward these (with four text figures)..........................LEWIS KNUDSON 430

No. 8, October

Taxonomic characters of the genera Alternaria and Macrosporium (with eleven text figures and plates XIX and XX) John A. Elliott 439
Crown-rot of fruit trees: histological studies (with plates XXI–XXVII) .... J. G. Grossenbacher 477

No. 9, November

Effect of soil temperature on the growth of bean plants and upon their susceptibility to a root parasite..... Donald Reddick 513
The development of Cortinarius pholideus (with plates XXVIII and XXIX) .... W. H. Sawyer, Jr. 520
Leaf-structure as related to environment (with six text figures) Herbert C. Hanson 533

No. 10, December

The phytogeography of Manoa Valley, Hawaiian Islands (with fourteen text figures) .... Vaughan MacCaughey 561
Revision of the Hawaiian species of the genus Cyrtandra, section Cylindrocalyces Hillebr. (with five text figures) Joseph F. Rock 604
On the distribution of abnormalities in the inflorescence of Spiraea Vanhouttei (with four text figures and plates XXX and XXXI) .... J. Arthur Harris 624
Index to Volume IV 637

(Dates of publication: No. 1, Feb. 3; No. 2, Feb. 17; No. 3, Mar. 23; No. 4, May 8; No. 5, May 31; No. 6, June 29, No. 7, July 14; No. 8, Oct. 2; No. 9, Nov. 24; No. 10, Dec. 12.)

ERRATA, VOLUME IV

Page 12, (") at end of paragraph 2 should be transferred to end of third line above, after the word **freezing**."

Page 13, insert **f** and **i** respectively as the first letters of the third and second lines from the bottom.

Page 371, for text *Fig. 2*, read **Fig. 3.**

Page 371, in explanation of figure, for *infrequens*, read **Fritillariae.**

Page 372, for text *Fig. 3*, read **Fig. 2.**

Page 372, in explanation of figure, for *Fritillariae*, read **Miurae.**

Vol. IV. JANUARY, 1917 No. 1

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CONTENTS

The influence of certain climatic factors on the development of Endothia parasitica (Murr.) And. . . . . NEIL E. STEVENS 1

Gunnera petaloidea Gand., a remarkable plant of the Hawaiian Islands. VAUGHAN MACCAUGHEY 33

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VOL. IV JANUARY, 1917 NO. 1

THE INFLUENCE OF CERTAIN CLIMATIC FACTORS ON THE DEVELOPMENT OF ENDOTHIA PARASITICA (MURR.) AND.

NEIL E. STEVENS

The chestnut blight is at present common from the northern limit of the chestnut, that is, southern New Hampshire and Vermont, to central Virginia. The area which it occupies includes the northern limits of growth of two native species of Endothia, *Endothia gyrosa* (Schw.) Fr. and *Endothia radicalis* (Schw.) De Not. It is also a transition region for several important plant diseases. In the southern portion of this territory bitter rot is one of the commonest and most destructive diseases of apples; in the nothern portion it is a botanical curiosity; and pear-blight, which is so abundant in the more southerly portions of this area, is hardly known from the northern states of New England. Apple scab, on the other hand, is more important in the northern portion than in the southern.

In order to gain more complete knowledge of the behavior of *Endothia parasitica* through this range and if possible to throw some light on the factors which limit the growth of these other fungi, the writer has undertaken a quantitative comparison of the growth and fructification of the fungus with the weather conditions, as far as data are available. While the work is not yet complete, enough data have accumulated to warrant the publication of results. This seems especially desirable in view of the fact that two of the stations, Wilmington, Delaware, and Hartford, Connecticut, must now be abandoned because of the general infection of the chestnut.

PLAN OF EXPERIMENTS

Previous observations on the growth and reproduction of *Endothia parasitica* have been confined chiefly to single localities, with little

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opportunity for comparison. Consequently in this work special care was taken to have inoculations made in the same way and on trees as nearly similar as possible but in different localities. It was desired of course to make observations at stations climatologically as different as possible. The actual location of inoculations was however governed by practical considerations. In order to avoid spreading the chestnut blight beyond its present range it was necessary to confine work to regions where the disease was so well established as to leave no hope of eradication. A quantity of healthy chestnut was obviously necessary. The latter consideration excluded the entire region between Philadelphia and New Haven, Connecticut. With the exception of one locality (Overlook Mountain) the inoculations were all made near regular U. S. Weather Bureau observation stations.[1]

The stations selected were Concord, N. H.; Williamstown and Amherst, Mass.; Hartford, Conn.; Wilmington, Del.; Van Bibber, Woodstock, and Frederick, Md.; Washington, D. C.; and Fairfax and Charlottesville, Va. The distance from Concord to Charlottesville is about 500 miles, or about 5 degrees of latitude. In addition to regular inoculations at these stations inoculations were made at various elevations on Overlook Mountain in the Catskills in order to determine whether difference in altitude would make any perceptible difference in the growth and fructification of *Endothia parasitica*. Overlook Mountain was selected as being the only place known to the writer where chestnut grows through a considerable range of elevation and where the chestnut blight is present. Graylock Mountain near Williamstown, Mass., was first selected but chestnut was not found on this mountain above 1,500 feet.

Work was begun in the spring of 1914 and each station visited once in five or six weeks during the summer of 1914 and twice during the summer of 1915. At each visit ten or more inoculations were made on healthy chestnut trees and the condition of previous inoculations noted. The trees inoculated were uniformly second growth and as far as possible were from 6 to 8 inches in diameter. Wherever these conditions were not met the fact is indicated in the report of observations. The inoculations were made by cutting through the bark with a sharp knife and inserting a quantity of mycelium and spores from a pure culture, usually on corn meal, with a freshly cut twig.

---

[1] In the selection of these stations, as well as in the interpretation of weather data, the writer had the advice of Mr. L. M. Tarr, local forecaster, U. S. Weather Bureau, New Haven, Conn.

Previous Work on Rate of Growth

Anderson (1, p. 16)[1] conducted experiments on the growth of *Endothia parasitica* on *Castanea dentata* at Charter Oak, Pa., during the summer of 1912, and Rankin (9) during the same summer at Napanoch, N. Y. Both these writers give the average growth for each month during the summer and Anderson gives it for the entire year. The average annual growth[2] at Charter Oak, Pa., for the year ending June 1, 1912, was 15.97 cm. according to Anderson (1, p. 575), while Rankin estimates 12 cm. about the average amount of a season's growth at Napanoch.

Rogers and Gravatt (10) made an intensive study of the spread of the chestnut blight over a small area near Bluemont, Va., and give 6.35 inches (15.87 cm.) as the average annual diameter growth of cankers at this point. They found the average growth on *Castanea pumila* near Leesburg, Va., for the year ending August 15, 1914, to be 6.8 inch (16.08 cm.). There is fairly close agreement among the results from Virginia and Pennsylvania even though they were taken in different years. The growth in New York is, however, considerably less.

Rate of Lateral Growth

Since *Endothia parasitica* kills its host by girdling the parts attacked, vertical growth is of no importance so far as its parasitic qualities are concerned, consequently in this work the rate of lateral growth alone was measured. As careful comparative measurements for various periods of the same year have already been given by Anderson (1) and Rankin (9), special attention was paid to determining the amount of growth for one year at the various points. On this account no cuts were made in the cankers until they were one year old. All measurements made previous to that time were taken from the sunken area in the bark.

Table I gives the annual lateral growth (determined by cutting away the bark) of cankers at the various stations for the years ending in May and in August, 1915, so far as the data are complete. Each figure represents an average of all the normal cankers; that is, cankers which developed only on one side of the cut were not included. These averages are expressed in the nearest centimeter, as that seems to the writer to represent about the degree of accuracy with which a number of cankers can be measured. These measurements are not exceptional in any way and in all probability represent about the average growth at those points during the year. In general, the growth for the year ending in May is about the same as that for the year ending in August. This is not true of inoculations made at all seasons however.

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[1] Reference is made by number to "Literature cited," p. 31.

[2] All measurements are for lateral growth.

Experiments during two seasons (1912–13 and 1913–14) indicate that inoculations of *Endothia parasitica* on Castanea made in the fall do not develop until the following spring. Those made in Maryland during November, 1912, showed no evidence of development until early in the following May. A similar series made early in November, 1913, showed no growth until spring and cankers from inoculations made in April, 1914, developed throughout the summer as rapidly as those made the fall before. These results agree with those of Anderson (1, p. 8) and Rankin (9, p. 244).

TABLE I

Lateral Growth of Cankers of Endothia parasitica in Various Localities

Locality	Elevation (in Feet)	Year Ending	Cm.	Year Ending	Cm.
		1915		*1915*	
Concord, N. H.	350	May 18	14	Aug. 19	14
Williamstown, Mass.	711 (900)	22	15	16	15
Amherst, Mass. (2 stations).	222	17	16	17	15
Hartford, Conn.	159 (350)	15	16	18	16
Woodstock, N. Y.	1,000	24	15	11	16
Wilmington, Del.	86	14	19	10	20
Van Bibber, Md.	100	14	20	Oct. 7	18
Woodstock, Md.	392	Apr. 27	20	Aug. 9	20
Frederick, Md.	275 (325)	27	23	9	(Sprout girdled. No records.)
Washington, D. C.	112 (300)	22	20	July 28	21
Fairfax, Va.	300	June 6	23	4	21
Charlottesville, Va.	854	Apr. 20	25	(Forest fire; no later records.)	

As is shown by the table, there is a more or less regular increase in the annual growth from Concord, N. H., to Charlottesville, Va. So great is this difference that it must obviously be due to the difference in climate and not to a variation in the trees. The record is unfortunately not complete at Frederick, Md., or Charlottesville, Va. At Frederick the trees inoculated in August proved too small and were girdled before the year was complete. At Charlottesville a forest fire destroyed the inoculated trees some time during the last week in April, 1915.

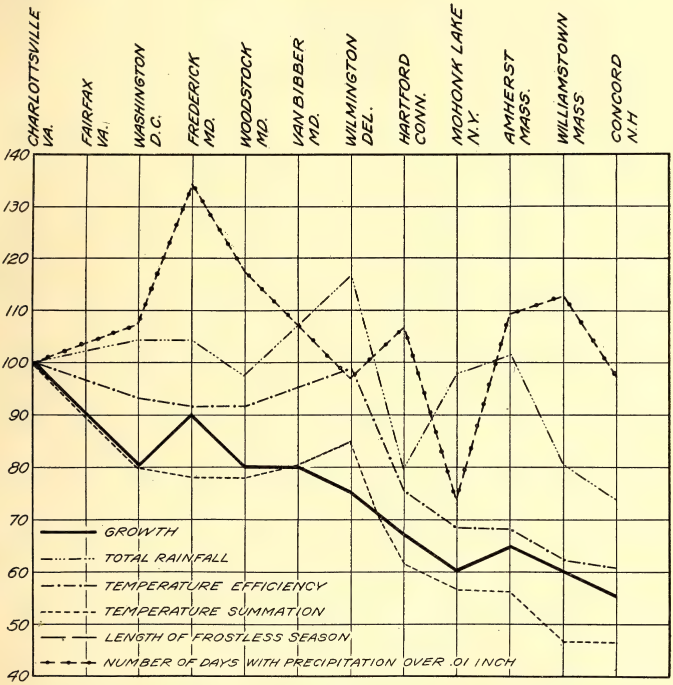

FIG. 1. Graphs showing the growth of *Endothia parasitica* on *Castanea dentata* and climatic data for the year ending April and May, 1915.

The relation of the amount of growth at the various stations is best seen from the curves (Figs. 1 and 2), where the amount of growth is expressed in percentage of that of Charlottesville. The amount at Charlottesville has been used as standard for comparison of all data in making curves, since this is the most southerly point and is near the center of the chestnut belt. This will also make comparisons easy in case points further south are studied as the chestnut blight advances.

For comparison with data just given the amount of annual growth for the years ending in May and August at various elevations on Overlook Mountain (Ulster Co., New York) is given (Table II). While the writer has no accurate data as to the temperature at these various elevations it is interesting and significant that in general

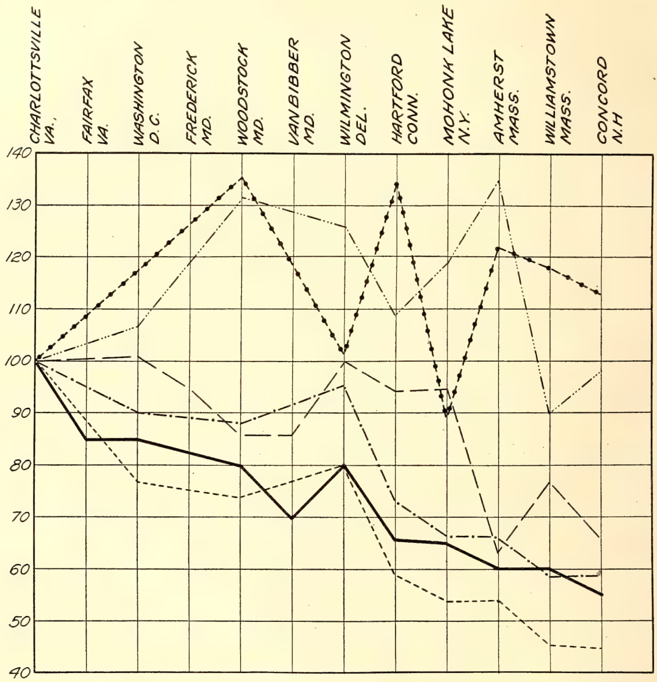

FIG. 2. Graphs showing the growth of *Endothia parasitica* on *Castanea dentata* and climatic data for the year ending August, 1915.

the amount of growth decreases with increased altitude. The only exceptions to this rule are in the case of Station O 4 which, being on the south side of the main ridge of Overlook, showed more growth than Stations O 2 and O 3 on the north side of the ridge. Station O 6 is also an exception to the general rule since though at an elevation of only 1,500 feet it showed but 10 cm. growth for a year. The writer is quite unable to explain this condition beyond the possibility that this reduced growth may be due to the fact that the trees inoculated were in a rather deep and shady ravine.

Table II

Lateral Growth of Cankers of Endothia parasitica at the Various Stations on Overlook Mountain, Woodstock, N. Y.

Station	Elevation (in Feet)	Year Ending	Cm.	Year Ending	Cm.
		1915		*1915*	
O 7	600	May 25	15	Aug. 11	15
S 3	1,000	26	15	11	16
S 1	1,500	24	14	11	15
C	1,500	23	14	11	
O 6	1,500	25	10	Aug. 12	10
O 1	1,900	27	14	12	13
O 2 (north side of ridge)	2,500	26	11	13	11
O 4 (south side of ridge)	2,800	26	13	13	11
O 3 (north side of range)	2,900	26	11	13	10

Climatological Data

In comparing the growth of this fungus with climatic conditions the highest degree of accuracy could be obtained only by carrying on a complete series of meteorological observations in each locality. This procedure, which would have required an observer stationed at each point, was impracticable. Consequently, it was decided to depend entirely on the data regularly furnished by the U. S. Weather Bureau. This, of course, necessitates neglecting certain factors known to be important to plant life. The writer is of the opinion, however, that if progress is soon to be made toward understanding the climatology of plant disease a serious attempt must be made to utilize the meteorological data already available.

While the climatic data available from the Weather Bureau records are not all that might be desired, all the stations except Van Bibber, Md., and Fairfax, Va., furnish daily maximum and minimum temperatures and amount of precipitation, as well as the number of clear, partly cloudy, and cloudy days, and the prevailing direction of the wind for each month. The date of last killing frost in spring and first

TABLE III

Total Precipitation.

	Concord, N. H.	Williamstown, Mass.	Amherst, Mass.	Hartford, Conn.	Mohonk Lake, N. Y.	Wilmington, Del.	Woodstock, Md.	Frederick, Md.	Washington, D. C.	Charlottesville, Va.
1914										
April	3.87	5.65	6.59	3.84	4.80	4.04	5.05	4.57	3.20	3.43
May	1.54	1.94	3.56	2.71	4.10	0.89	1.54	2.53	1.72	1.48
June	2.39	2.21	2.32	1.70	2.40	2.95	2.77	3.87	6.20	2.38
July	3.49	4.33	3.53	4.30	3.75	7.17	1.28	2.96	2.32	2.67
August	5.91	5.10	5.11	1.96	2.54	3.13	6.80	5.96	6.00	2.35
September	0.21	0.53	0.52	0.20	0.32	0.86	1.02	1.41	0.66	1.17
October	1.12	1.72	2.09	3.05	3.55	2.19	1.50	1.70	1.65	5.16
November	2.27	2.14	2.62	2.38	3.54	1.75	1.96	1.81	2.06	2.96
December	2.07	1.94	2.89	3.85	3.93	5.90	4.35	4.28	4.49	5.37
1915										
January	3.47	3.45	6.52	5.70	6.56	5.83	7.75	7.35	6.34	5.90
February	2.89	4.14	7.02	4.30	4.46	6.21	4.84	5.06	3.60	4.91
March	Trace	0.41	0.12	0.29	0.28	1.17	1.38	1.07	1.07	0.95
April	2.62	2.12	3.99	1.58	2.01	3.15	1.52	0.70	0.90*	0.49
May	0.99	1.46	1.20	2.53*	2.54*	4.88*	3.86*	4.21*	2.18	2.44
June	1.39	1.73	3.00	1.51	2.65	6.04	6.20	7.14	6.58	5.32
July	10.29	9.37	9.13	6.97	8.24	2.39	3.29	3.59	3.21*	3.71
August	6.26*	4.47*	8.28*	6.83	7.94	6.32	8.77	9.49	7.00	7.83
September	....	....	....	....	....	....	....	....	1.39*	
Total for year ending	May 18 27.04	May 22 29.64	May 17 37.36	May 15 29.30	May 24 35.73	May 14 42.41	Apr. 27 35.96	Apr. 27 38.34	Apr. 22 38.16	Apr. 22 36.78
Total for year ending	Aug. 19 35.68	Aug. 16 33.97	Aug. 17 49.83	Aug. 18 40.22	Aug. 11 43.66	Aug. 10 46.39	Aug. 9 48.76	....	July 28 39.59	....

* An asterisk indicates the month in which ascospores were first observed at the various stations.

TABLE IV

Number of Days with Precipitation 0.01 Inch or More

	Concord, N. H.	Williams-town, Mass.	Amherst, Mass.	Hartford, Conn.	Mohonk Lake, N. Y.	Wilmington, Del.	Woodstock, Md.	Frederick, Md.	Washing-ton, D. C.	Charlottes-ville, Va.
1914										
April	14	14	16	17	8	11	12	11	9	8
May	6	9	11	7	3	5	6	8	7	5
June	8	10	8	12	7	10	14	12	11	11
July	11	13	14	14	10	16	7	12	12	8
August	12	11	10	11	6	9	12	13	10	11
September	4	3	6	4	2	2	5	9	4	5
October	6	5	5	5	2	4	9	7	7	9
November	8	11	7	7	7	2	3	4	4	4
December	10	8	8	9	7	11	17	15	12	16
1915										
January	10	11	12	13	7	13	12	11	14	9
February	8	9	12	11	5	8	8	10	8	7
March	0	6	3	3	2	2	5	7	5	4
April	8	9	7	7	6	9	10	18	7	4
May	9	8	11	11	9	14	16	13	11	10
June	10	11	8	13	7	11	13	12	14	10
July	16	14	20	16	18	7	13	15	13	12
August	15	15	14	13	10	11	15	18	18	15
Total for year ending	May 18 91	May 22 104	May 17 102	May 15 101	May 24 69	May 14 92	Apr. 27 109	Apr. 27 124	Apr. 22 101	Apr. 22 93
Total for year ending	Aug. 19 106	Aug. 16 110	Aug. 17 114	Aug. 18 127	Aug. 11 82	Aug. 10 94	Aug. 9 126	....	July 28 109	....

killing frost in autumn is also available for most of the stations and the regular Weather Bureau observation stations give the percentage of possible sunshine each day as well as atmospheric pressure and direction and velocity of wind.

Among the climatic elements recorded, any direct relation between atmospheric pressure and growth is very difficult to trace. Wind velocity and light while undoubtedly important for a green plant probably have little relation to the growth of *Endothia parasitica*, especially since the advancing edge of the mycelium is under the unbroken bark of the host tree. A careful study of the Weather Bureau data shows no correlation between amount of growth and either the prevailing direction of the wind or the number of clear days during the period investigated. The writer's laboratory experiments also have failed to demonstrate any relation between the amount of light and the growth and fructification of the fungus even when growing on the surface of culture media.

PRECIPITATION

As regards precipitation, there are plainly two elements to be considered: the amount of rainfall and its distribution. Table III gives the monthly precipitation for each station during the course of the investigations, Table IV the number of days with more than .01 inch precipitation for each year during the same period. Careful examination of rainfall data fails to show any relation between either amount or frequency of rain and the amount of growth of the fungus. Amherst, Mass., had practically the same rainfall as Charlottesville, Va. Yet the growth at the latter point averaged nearly four inches greater. Williamstown, Mass., on the other hand, had a much smaller rainfall than Amherst but showed about the same amount of growth. Amherst, Mass., and Hartford, Conn., had much greater rainfall for the year ending in August than for that ending in May, yet the amount of growth was practically the same.

While the different localities show considerable variation both in the amount of rainfall and in the number of days with rain this seems to have no relation to the amount of growth. This is probably best shown by the curves (figs. 1 and 2) of rainfall and number of days with precipitation. The various points of these curves are expressed in percentage of the rainfall and number of days with rain at Charlottesville, Va. The irregularity of the rainfall curves as compared with the curve of growth makes it seem almost impossible that either total amount of rainfall or number of days with rain has any direct effect on the growth of the fungus. This is theoretically very probable since the growing edge of the fungus is in or near the cambium of the host under the bark and its moisture supply must come from the host itself.

It is conceivable that a fungus might be susceptible to changes in the water content of those portions of its host in which it grows, so slight as not to produce a perceptible effect on the host. There is, however, no evidence that such is the case in *Endothia parasitica*. On the other hand Rankin (9, p. 245) who investigated the relation of the growth of *Endothia parasitica* to the water content of the bark of *Castanea dentata* during the summer of 1912 at Napanoch, N. Y., failed to demonstrate that the "variation in the physiology of the tree which results from drought conditions alters to any great degree either the susceptibility of the chestnut tree or the rate of progress of the mycelium in the bark."

With his conclusion the writer's observations entirely agree. In the course of three years' inoculation experiments and field observation the writer has been unable to obtain any evidence that the rate of growth of this fungus is affected by external dryness which does not produce a perceptible withering effect on the host.

Length of Frostless Season

The only remaining factor seems to be that of temperature. Zon (13) has emphasized the necessity of considering the length of the growing period in plant climatology and the advisability of tabulating climatic data separately for the period of growth and the period of rest. While his contention is undoubtedly correct for green plants it is apparently not true in the case of *Endothia parasitica* which has, strictly speaking, no resting season. Field observations and laboratory experiments both show that *Endothia parasitica* will grow whenever the temperature rises above its minimum for growth, which, as Shear and Stevens (11, p. 7) have determined, is about 8° or 9° C. This is apparently true regardless of the previous temperature and whether the host is dormant or not.

Anderson and Rankin (3, p. 574) conducted experiments separately at Charter Oak, Pa., and Napanoch, N. Y., and agree that the chief growth of *Endothia parasitica* occurs between March and October but that the fungus continues to grow during mild periods of winter. During January, 1913, an average lateral growth of 0.51 cm. was recorded for cankers at Charter Oak, Pa., while no growth whatever was recorded in November, December, or February. In this connection Anderson and Rankin call attention to the fact that during January there were ten different days in which a temperature above 9° C. was recorded. As Rankin (9, p. 244) states, "cessation of growth of the mycelium in the bark during fall and winter as well as negative results of inoculations at this time of the year is explained purely on the basis that the temperature is too low for the vegetative activity of the fungus."

Experiments made by the writer with plate cultures of *Endothia parasitica* in the laboratory agree with these field observations. When such cultures were kept at temperatures below their minimum for growth, that is, 7° C., 3° C., and 1° C., for twenty-four hours and then removed to room temperature for twenty-four hours, they grew practically as much while in the warm room as did cultures which had never been in the ice box. So quickly does the fungus recover from the effect of the low temperatures that plate cultures which were kept in the refrigerator for twenty-two hours and at room temperature for only two hours each day showed a measurable growth at the end of a week. Spring weather, with warm days and cool nights or even a warm period in midwinter would then permit growth. In fact plate cultures kept out doors at Washington, D. C., during January, 1915, made a total growth of 1.5 cm. for the month. Anderson and Rankin further state (3, p. 575) that "the mycelium does not seem to be injured in the least by freezing, but remains alive in all parts of the canker during the winter. These investigators report that cultures kept frozen for a month at a time renewed growth naturally on being brought back into the laboratory."

This being the case one would expect to find little connection between the length of frostless season and the amount of growth in the various localities. Table V gives the length of frost-free period in days during the time of the experiment at the various stations. There is of course in general a decrease in the length of frost-free period from Charlottesville northward. This is, however, not regular, since the length of frost-free period is greater at Hartford, Conn., than at Van Bibber or Woodstock, Md., while the growth is of course greater at the latter points. Williamstown, Mass., had a considerably shorter frost-free period than Hartford, Conn., and on the other hand a much longer frost-free period than Amherst, Mass., while the amount of growth at these points is practically the same. The curves, figure 2, in which the length of frost-free period at the various points is indicated in percentage of the period at Charlottesville, show that while there is in general a falling off in the length of frost-free period from Charlottesville to Concord, the agreement between this curve and the curve of growth is not such as to indicate any direct causal relation.

TABLE V

Frost Data for Various Localities

Locality	First Killing Frost in Autumn, 1914	Last Killing Frost in Spring, 1915	Length of Frost-free Period in Days
Concord, N. H.	September 29	May 15	137
Amherst, Mass.	28	20	131
Williamstown, Mass.	29	April 22	160
Hartford, Conn.	October 27	13	197
Mohonk Lake, N. Y.	14	14	183
Wilmington, Del.	28	4	207
Van Bibber, Md.	September 29	15	167
Woodstock, Md.	29	15	167
Frederick, Md.	October 28	15	196
Washington, D. C.	28	3	208
Charlottesville, Va.	28	5	206

TEMPERATURE

In measuring the effectiveness of temperature in plant climatological studies annual or monthly means are obviously of very little significance. As has been frequently pointed out, localities with similar mean annual temperatures may have actually very different climatic conditions. Among the methods of measuring temperature more satisfactorily probably the most used is that of direct summation of daily mean temperatures. Merriam (6) was the first to apply this method in preparing a chart of the climatic zones of the United States. Briefly, the method is as follows: A certain minimum temperature is assumed as a starting point and the amount added to the summation each day is the number of degrees above the assumed minimum which represents the mean temperature for that day. The minimum is sometimes the freezing point but often a somewhat higher temperature.

Recently the Livingstons (5, p. 353) have called attention to the fact that although these temperature summations have in many instances furnished data consistent among themselves and constituting an apparently reliable criterion for the measurement of the intensity and duration aspects of the temperature factor it is improbable that any fundamental or general principle regarding the influence of temperature in a plant is derived from the relations thus brought out. They suggest as more satisfactory for measuring temperature effectiveness a method of calculating temperature efficiencies based on the well-known chemical principle of van't Hoff and Arrhenius, that within limits the velocity of most chemical reactions doubles or somewhat more than doubles for each rise in temperature of 10° C. On this basis the Livingstons (5, p. 366) have prepared a table of approximate efficiency indices for temperatures in whole degrees from 40° F. to 99° F., assuming the efficiency to be unity at 40° and to double with each rise in temperature of 18°, and have prepared maps of the United States comparing temperature summations with the temperature efficiencies calculated according to their tables. The results of the two methods show a rather close general agreement but there are numerous discrepancies in detail.

For purposes of comparison both methods have been used in the present work. In all calculations the mean for each day was determined by the formula: Mean = $\frac{1}{2}$(maximum + minimum). The calculations have been made in the Fahrenheit scale, not because this scale is as convenient as the Centigrade but because all Weather Bureau data are so published.

Direct Temperature Summations

The direct temperature summations have been calculated for all the stations where complete data are available.[4] Forty-five degrees F. has been regarded as the zero point, since it is undoubtedly slightly below the temperature at which *Endothia parasitica* is able to grow (11). The amount added each day is then one half the sum of the maximum plus the minimum temperature as given in the monthly reports of climatological data issued by the Weather Bureau. The sum of these amounts for the 365 days for which the growth of the canker was measured is the temperature summation for the year. Table VI gives these summations for the various localities and the percentage of each when the summation at Charlottesville for the year ending April 23, 1915, is considered 100 percent.

With the single exception of Wilmington there is a fairly regular decrease in the temperature summations from Charlottesville northward. A comparison of the curves of growth and temperature summation (figs. 1 and 2) shows that there are some irregularities and that the temperature summation falls somewhat more rapidly northward than does the amount of growth.

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[4] The writer is indebted to Mr. Anthony Merryman for much assistance in calculating weather data.

Table VI

Temperature Summations

Locality	Year Ending	Summation	Percent	Year Ending	Summation	Percent
Charlottesville, Va....	Apr. 20	6,412	100.00			
Washington, D. C....	22	5,153	80.0	July 28	4,941	77.1
Frederick, Md........	27	5,005	78.1			
Woodstock, Md.......	27	5,024	78.3	Aug. 9	4,742	73.8
Wilmington, Del......	May 14	5,443	84.9	10	5,169	80.0
Mohonk Lake, N. Y. .	24	3,623	56.5	11	3,465	54.0
Hartford, Conn.......	15	3,943	61.0	18	3,779	59.0
Amherst, Mass........	17	3,584	55.9	17	3,479	54.3
Williamstown, Mass. .	22	3,017	47.0	16	2,970	46.3
Concord, N. H.......	18	3,045	47.5	19	2,924	45.6

Temperature Efficiencies

In calculating temperature efficiencies the Livingstons' method was adopted with no change except in the zero point. That is, it was assumed that the efficiency doubled with each rise in temperature of 18° F., since this assumption seems to agree most nearly with the work of the numerous investigators who have sought to determine the application of the van't Hoff-Arrhenius principle to physiology.[5]

There is, of course, no direct evidence that growth in the case of this particular fungus is accelerated by rise in temperature at the rate assumed. The calculations were made rather to determine how closely the general law would apply to this organism under field conditions.

Efficiency was assumed to be unity at 45° F. The writer is however inclined to believe now that 47° might be even more accurate. This makes the formula for calculating efficiency

$$e = 2^{\frac{t-45}{18}},$$

when e = the efficiency and t the daily mean temperature. A table was prepared on this basis and used in calculating the temperature efficiency for each day. The table is obviously the same as that given by the Livingstons (5, p. 366) except that it assumes 45° instead of 40° to equal unity. Whenever the mean daily temperature was below 45° the efficiency was considered zero. The efficiency index of each locality for a year is the sum of the daily indices.

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[5] For a brief résumé of the literature on this point see 5, p. 356–359.

Table VII gives the temperature efficiencies for the various localities studied and the percentage of each based on the temperature efficiency of Charlottesville as 100 percent. This table should be compared first of course with the table of temperature summations. As the figures of the efficiency index at Charlottesville approximately equal the first two figures of the temperature summation at that point a rough direct comparison is possible. In general, it is evident that the temperature efficiency indices fall off less rapidly in amount from Charlottesville northward than do the temperature summations. This is shown more strikingly by the percentages and as is indicated by the figures the curve of temperature efficiency follows the curve of growth more closely for the northern localities than does the curve of temperature summations. The former falls slightly less rapidly than does the growth curve; the latter somewhat more rapidly. The only serious exception is Wilmington which has higher temperature summation and efficiency indices than the other Maryland stations or even Washington, D. C., without a corresponding increase in amount of growth. This discrepancy the writer is wholly unable to explain.

TABLE VII

Temperature Efficiencies

Locality	Year Ending	Efficiencies	Percent	Year Ending	Efficiencies	Percent
Charlottesville, Va....	Apr. 20	635	100.0	....	....	....
Fairfax, Va..........	....	....	....	....	....	....
Washington, D. C....	Apr. 22	594	94	July 28	574	90
Frederick, Md.......	27	586	93	....	....	....
Woodstock, Md......	27	586	92	Aug. 9	562	89
Van Bibber, Md......	....	....	....	....	....	....
Wilmington, Del......	May 13	632	99	Aug. 10	605	95
Hartford, Conn.......	15	481	76	18	463	73
Mohonk Lake, N. Y..	24	435	68	11	421	66
Amherst, Mass.......	17	431	68	17	421	66
Williamstown, Mass. .	22	396	62	16	381	60
Concord, N. H.......	18	384	60	19	371	58

When the extent of the territory covered and the necessarily approximate nature of the data and their calculation are considered the degree of correlation between the curves of growth and of temperature is remarkably close. In general the correlation is slightly less perfect when the effect of temperature is expressed by efficiency indices than by direct summation. In either case, there can be little doubt that under climatic conditions in which the optimum temperature of the fungus is rarely greatly exceeded (11, p. 9) the amount of growth made by *Endothia parasitica* depends directly on the amount and duration of heat available. If this conclusion is correct the chestnut blight may be expected to spread somewhat faster in the future than it has in the past unless other factors intervene to check its growth. For instance, the temperature summation for Corinth, Miss. (year ending June 1, 1915), where there is still some chestnut and where *Endothia fluens mississippiensis* was first collected, is 6,561 or 102.0 percent of the summation at Charlottesville. The efficiency at that point is 764 or 120.3 percent of that at Charlottesville. The chestnut blight should then be able to make at Corinth a growth somewhat greater than that at Charlottesville and considerably greater than that at any of the northern points.

At first glance the statement that the amount of lateral growth of *Endothia parasitica* is dependent directly on temperature may seem so simple an explanation as to be artificial. A consideration of the conditions under which the advancing edge of the mycelium lives in the host shows, however, that the biological conditions are unusually constant and that the fungus is very little influenced by many factors of great importance to green plants.

The environmental factors most used in such a classification of plants as that given by Köppen (4), for instance, are many of them negligible. The chemical nature of the medium in which the fungus grows parasitically must be fairly constant since it is always the same portion of the same host species. Certainly the difference between individual trees of this species is so slight that as yet no tree resistant to this fungus has been found.

Light, so important in the growth of green plants, is negligible here. The writer has thus far been unable to demonstrate that light had any effect on the growth or reproduction of this fungus under laboratory conditions and in all probability no light whatever reaches the advancing edge of the mycelium under the bark.

The fungus has, moreover, no resting season. It is almost independent of external moisture supply since it lives in the portion of the host where moisture is most abundant, and where evaporation is very slight, if indeed it occurs at all.

If the biological relations of the fungus are correctly understood it is, while growing as a parasite in or near the cambium of its host, uninfluenced by any environmental condition except that of temperature, at least in the territory it now occupies in this country. And the influence of temperature itself is restricted to an increase or decrease of the amount of growth rather than any permanent cessation of growth such as is brought about by heavy frost in the case of green plants.

Ascospore Production

In studying the relation of climatic conditions to reproduction in *Endothia parasitica* attention was concentrated on the production of ascospores. The time necessary for the development of pycnidia is so short that to determine the factors involved would necessitate an intensive study of a few adjacent localities, with much more frequent visits than were possible in covering so large an area as was involved in the present study.

Previous observations on the production of ascospores have been isolated rather than comparative. Murrill (8, p. 187), in his original description of the fungus, stated: "The winter spores [ascospores] mature in late autumn . . . and germinate the following spring."

Anderson and Babcock (2, p. 36) made several hundred inoculations on various dates from May 29 to July 12, 1912, and recorded the date of appearance of pycnospore horns and perithecia. They conclude that (p. 37): "In general it may be said that under natural conditions in the summer time the spore horns will be developed in from three to six weeks, and that the winter or ascospore stage will develop in ten weeks or more. The fact that the perithecial stage on all these plots appeared in September and October should not be interpreted as indicating that the approach of winter had any influence in bringing about this stage."

Rankin (9, p. 249) made inoculations at Napanoch, Ulster Co., N. Y., each month during the summer of 1912 commencing with May and observed that stromata were not produced on any of the cankers until about the second week of September (p. 254), and that they appeared as quickly on cankers produced by inoculations of July 4 as on those made May 1. Cankers produced from inoculations made at different times from May 1 to August 1 showed uniformly mature perithecia and ascospores by the middle of November. He refers to the perithecial stromata developing "abundantly in the autumn around the old pycnidia."

Rogers and Gravatt (10, p. 45) report that in their inoculations at Leesburg, Va., made on July 21 and August 16, 1912, pycnidia with spore horns were developed by October 6. Although the cankers were examined in March and again in August, 1914, no perithecia were found.

That unfavorable conditions may delay for a long time and perhaps entirely prevent the production of ascospores was first brought to the writer's attention by inoculations of *Endothia parasitica* on chestnut sprouts near Washington, D. C. These inoculations were made in July, 1913, and produced abundant pycnidia within two weeks. Sections of the stromata made in September, 1913, showed numerous fundaments of perithecia. The inoculations were conveniently located and as they were from the first material sent from China by Meyer were frequently examined. The cankers continued to grow normally and in most cases girdled the sprouts and formed numerous stromata with abundant pycnospores and fundaments of perithecia. Up to December, 1914, however, when the sprouts were destroyed by fire, no ascospores had developed.

Field Observations

When this work was begun it was expected that ascospores would be produced in the fall as had been the case in the work of Anderson and Babcock and of Rankin and other investigators. Actually, however, at none of the stations was a single canker in the entire series of inoculations found which had produced ascospores or even mature appearing perithecia during the season for 1914. In 1915, however, quite different climatic conditions existed. Perithecia and mature ascospores were found in abundance not only on cankers arising from inoculations made in 1914 but from those made in May, 1915. The problem then became not so much a comparison of the fructification at different localities as a comparison of the fructification during different seasons at the same locality.

Table VIII gives the results of observations at the various localities on the development of perithecia and mature ascospores. It is evident that no perithecia were produced during the season of 1914 at any of the localities. Observations made December, 1914, at stations as

Table VIII

Observations on the Development of Perithecia and Mature Ascospores at Various Localities

Locality	Inoculations Made	No Perithecia Up to and Including	Perithecia with Mature Ascospores First Observed	Additional Notes
Charlottesville, Va.	Apr. 20, May 21, July 3, Aug. 11, and Oct. 2, 1914.	Dec. 24, 1914.	....	Destroyed by fire last week in Apr., 1915.
Fairfax, Va.	Apr. 21, June 6, July 4, Aug. 1, and Oct. 24, 1914.	Dec. 23, 1914.	June 6, 1915. On cankers from all inoculations except those of Oct. 24, 1915 and Aug. 6, 1915, from inoculations of Oct. 24, 1915.	One of the trees inoculated Apr. 21, 1914, died during that summer and no ascospores were produced on this tree.
Vienna, Va.	Apr. 2, May 14, June 6 and July 18, 1915.	Aug. 1, 1915.	Sept. 21, 1915. Abundant from all inoculations.	Perithecia more abundant on inoculations of June 6 and July 18 than previous ones.
Washington, D. C.	Apr. 22, May 28, June 25, July 28, and Oct. 21, 1914.	Dec. 25, 1914.	A few mature perithecia, Apr. 22, 1915, from inoculations except those of Oct. 21, 1914. Abundant July 28, 1915, on all.	....
Frederick, Md.	Apr. 27, May 30, Aug. 9, and Oct. 19, 1914.	Dec. 20, 1914.	May 14, 1915. A few from inoculations of Apr. 27 and May 30, 1914.	In 1914 there was less development of pycnospore horns from May 30 to Aug. 9 than in the month preceding May 30.
Woodstock, Md.	......do......	Dec. 27, 1914.	Aug. 9, 1915. Numerous on all inoculations.	
Van Bibber, Md.........	Apr. 28, May 14, June 1, July 6, Aug. 10, Oct. 7, 1914; May 14, 1915.	Dec. 28, 1914.	May 14, 1915, from all inoculations except those of Oct. 7, 1914. Oct. 7, 1915, from all inoculations, including those of May 14, 1915.	....
Wilmington, Del.	Apr. 29, May 14, June 1, July 6, and Aug. 10, 1914.	Dec. 28, 1914.	May 14, 1915. Numerous from inoculation of April 29. Few from inoculations of May, June, and July. Aug. 10, 1915. Numerous on all mentioned above.	No perithecia were developed from the inoculations made Aug. 10, 1914, but the number from the earlier inoculations were greatly increased.

TABLE VIII—(*Continued*)

Locality	Inoculations Made	No Perithecia Up to and Including	Perithecia with Mature Ascospores First Observed	Additional Notes
Hartford, Conn.	May 15, June 8, July 15, Aug. 18, and Sept. 24.	Sept. 23, 1914.	May 15, 1915. A few mature perithecia. Aug. 18, 1915. Abundant from all inoculations.	....
Amherst, Mass.	May 17, June 8, July 15, Aug. 17, Sept. 24, 1914; May 17, 1915.	May 17, 1915.	Aug. 17, 1915. From all inoculations, including those of May 17, 1915.	....
Williamstown, Mass.	May 22, June 9, July 14, Aug. 16 and Sept. 25, 1914, and May 22, 1915.	May 22, 1915. Occasional immature perithecia were found on this date.	Aug. 16, 1915. Numerous from all inoculations except those of May 22, 1915.	The trees inoculated May 22, 1915, had unusually thick bark.
Concord, N. H.	May 18, June 11, July 17, Aug. 20, and Sept. 22, 1914, and May 18, 1915.	May 18, 1915.	Aug. 19, 1915. Present in cankers from inoculations on all dates, including those of May 18, 1915.	....

Locality		Inoculations Made	No Perithecia Up to and Including	Perithecia with Mature Ascospores First Observed	Additional Notes
Stations on Overlook Mountain.	Elevation in Feet				
O 7	600	On or about May 25, June 12, July 10, Aug. 12, and Oct. 1, 1914.	Oct., 1915.	May 15, 1915. Abundant.	....
S 3	1,000	Do.	Oct. 1, 1915.	May 24, 1915. Abundant near the center of one canker. Aug. 14, 1915. Abundant in all cankers.	....
S 1	1,500	On or about May 25, June 12, July 10, Aug. 12, and Oct. 1, 1914. and May 25, 1915.	May 24, 1915.	Aug. 11, 1915. Abundant on all, including inoculations of May 24, 1915.	Old cankers which had perithecia were producing pycnospore tendrils in large quantities often in the same stromata.

TABLE VIII (*Continued*)

Locality		Inoculations Made	No Perithecia Up to and Including	Perithecia with Mature Ascospores First Observed	Additional Notes
Stations on Overlook Mountain	Elevation in Feet				
C	1,500	On or about May 25, June 12, July 10, Aug. 12, and Oct. 1, 1914.	May 23, 1915.	Aug. 11, 1915. Present in cankers from all inoculations.	Perithecia less numerous than at the lower stations.
O 6	1,500	Do.	Oct. 1, 1915.	May 26, 1915. Present in cankers from inoculations of May and June 1915. Aug. 11, 1915, abundant in all.	....
O 1	1,900	Do.	May 26, 1915.	Aug. 13, 1914. Abundant.	....
O 2	2,500	Do.	Aug. 12, 1915.	....	....
O 4	2,800	On or about May 25, June 12, July 10, Aug. 12, and Oct. 1, 1914. and May 25, 1915.	May 26. Nearly mature.	Aug. 13. Mature perithecia from one inoculation of May 14.	....
O 3	2,900	On or about May 25, June 12, July 10, Aug. 12, and Oct. 1, 1914.	Aug. 13, 1915.	....	....

far north as Wilmington failed to show any perithecia. Perithecia did, however, develop during the late winter and spring as far north as Hartford, Conn., and up to an altitude of 1,500 feet on Overlook Mountain. Perithecia developed also at both northern and southern stations during the summer of 1915 although they were somewhat less abundant at Wilmington than at other stations and were found at only one of the three highest stations on Overlook Mountain and here only rarely.

TEMPERATURE

On comparing these data (see Table VIII) with the Weather Bureau records it is evident that perithecia may be produced under quite different temperature conditions. In our investigations they were produced between December 25 and April 22 at Washington,

D. C., at Woodstock, Md., Van Bibber, Md., and Wilmington, Del., and they were developed during the period between December 26 and February 15 at Washington Junction, Md. They were also produced in small number at an elevation of 1,000 feet on Overlook Mountain between October 1 and May 2. On the other hand, perithecia have been produced in mid-summer at all stations from Concord, N. H., to Vienna, Va. Perithecia were not produced during the winter or spring north of Hartford, Conn., and low temperature may in this case have been a limiting factor. Certainly perithecia are developed through a considerable range of temperature.

Table IX

Monthly Temperature Efficiency Indices for Various Localities

	Charlottesville, Va.	Washington, D. C.	Frederick, Md.	Woodstock, Md.	Wilmington, Del.	Hartford, Conn.	Mohonk Lake, N. Y.	Amherst, Mass.	Williamstown, Mass.	Concord, N. H.
1914										
May	85	76	75	73	..	..	..	..	..	28
June	89	94	96	93	95	71	66	66	63	60
July	107	106	103	101	106	82	75	79	75	75
August	117	98	102	103	109	87	79	82	74	73
September	76	72	67	68	79	65	65	58	51	57
October	57	56	52	57	61	49	46	43	38	37
November	28	25	22	22	27	15	10	9	7	6
December	4	6	5	7	5	3	4	1	4	0
1915										
January	3	5	3	5	4	3	0	0	1	0
February	10	9	4	6	10	3	0	0	1	0
March	6	1	3	2	5	2	0	0	0	0
April	61	56	55	52	52	36	39	36	33	29
May	..	63	..	61	62	47	44	45	42	39
June	..	84	..	77	80	68	63	65	64	58
July	..	105	..	98	110	86	77	82	76	77
August	..	98	..	95	100	80	71	76	67	71

It has been rather generally believed that low temperature was a determining factor in the production of ascospores by pyrenomycetes and ascospores have often been loosely referred to as "winter spores," a term used indeed in connection with *Endothia parasitica* (8, p. 187). That low temperatures are not necessary for the production of ascospores by *Endothia parasitica* is shown by the fact that they developed before September 21, 1915, from inoculations made July 18, 1915, at Vienna, Va., during which time no temperature below 54° was recorded, and the mean temperature was well over 70°. That high temperatures on the other hand are not necessary is shown by the fact that ascospores developed at many stations between December, 1914, and May, 1915, and at Washington Junction, Md., between December 26, 1914, and February 15, 1915. Certainly (see Tables IX and X) the difference between the summer temperatures of 1914 and of 1915 is so slight that the failure of perithecia to develop in the first summer and their abundance in the second summer cannot be due to the difference in temperature.

TABLE X

Monthly Temperature Summations for Various Localities

	Charlottesville, Va.	Washington, D. C.	Frederick, Md	Woodstock, Md.	Wilmington, Del.	Hartford, Conn.	Mohonk Lake, N. Y.	Amherst, N. Y.	Williamstown, Mass.	Concord, N. H,
1914										
May	765	717	663	639	716	540	489	439	408	345
June	921	870	870	858	880	618	599	590	557	539
July	1,000	960	952	934	970	757	702	737	707	684
August	1,048	892	931	952	998	825	745	770	676	676
September	672	643	584	591	712	541	549	488	404	441
October	480	481	432	443	507	341	347	313	74	232
November	188	138	100	125	143	67	26	29	18	23
December	25	36	31	36	29	8	14	3	11	0
1915										
January	8	18	6	12	17	10	0	0	2	0
February	40	33	12	19	35	1	0	0	2	0
March	18	4	2	5	11	3	0	0	0	0
April	501	449	432	430	426	228	281	228	238	163
May	604	549	512	523	539	338	281	290	232	198
June	760	774	754	718	736	640	561	590	591	514
July	993	973	963	869	1,014	807	716	779	727	720
August	899	905	881	885	923	743	641	703	622	644

MOISTURE

There seems, however, to be a fairly constant relation between the appearance of perithecia and the amount of precipitation, or more properly the amount of moisture in the air. For convenience in reference a * has been placed in Table III to indicate the month in which ascospores were first observed at the various stations. At many localities perithecia were first noted in the spring, a season which of course is characterized by high humidity. In each case in which ascospores were produced during the summer the preceding months were characterized by abundant rainfall. July, 1915, at Concord, Williamstown, and Amherst, showed over 9 inches of rain and the perithecial production was correspondingly abundant.

OBSERVATIONS IN ULSTER COUNTY, NEW YORK

Perhaps the most complete records regarding the appearance of perithecia and ascospores are the inoculations at Ulster Co., New York. As stated above, Rankin (9) found at Napanoch, N. Y., that ascospores were produced by the middle of November from inoculations made at different times from May 1 to August 1.

TABLE XI

Monthly Climatological Data for Three Seasons at Mohonk Lake, N. Y.

	Temperature			Precipitation	
	Mean	Summation	Efficiency	In Inches	Days with Over .01 Inch
1912.					
May..........	58.6	440	54	3.99	11
June..........	65.6	626	70	1.30	4
July...........	70.6	802	86	3.42	11
August........	64.8	624	67	3.88	12
September.....	60.8	475	56	3.28	14
October........	53.9	...	...	4.50	8
1914.					
May..........	61.2	489	...	4.10	3
June..........	64.8	599	66	2.40	7
July...........	67.0	702	75	3.75	10
August........	68.8	745	79	2.54	6
September.....	63.2	549	65	0.32	2
October........	55.7	347	46	3.55	2
1915.					
May..........	53.6	281	44	2.54	9
June..........	63.5	561	63	2.65	7
July...........	68.2	716	77	8.24	18
August........	65.4	641	71	7.94	10

The writer made a somewhat similar series of inoculations during the summer of 1914 at Woodstock. Inoculations were made each month in ten different localities on Overlook Mountain. None of these produced perithecia during the season of 1914, but most of them as well as inoculations made in May, 1915, produced perithecia abundantly by the middle of August, 1915. As Rankin made over 1,500 inoculations and the writer made more than twice that number the results were probably not due to chance but to a difference in the weather conditions.

The nearest weather station to these two localities is at Mohonk Lake, in Ulster County, elevation 1,245 feet. Mohonk Lake is between Napanoch and Woodstock, about equidistant from them and has about the same elevation. Observations made at this point while not absolutely identical with conditions at either of the stations would undoubtedly approximate the conditions at both. This was certainly true in the seasons under consideration for the Monthly Weather Reports of that section indicate that the weather conditions recorded at Mohonk Lake prevailed generally over the Eastern Plateau region.

Table XI gives the monthly precipitation, monthly mean temperature, temperature efficiency, and temperature summation, for the growing seasons of 1912, 1914, and 1915, at Mohonk Lake, N. Y. Comparison of the data for the three seasons shows only slight differences in temperature. June and July were warmest in 1912, August and September warmest in 1914. These differences are, however, slight, and can hardly have been significant in preventing ascospore production in 1914, since ascospores have been produced elsewhere at higher as well as lower temperatures.

There is on the other hand a considerable difference in the precipitation of the three years. 1915, when ascospores were produced abundantly before August 15, had much heavier rainfall in July than either of the other years. In 1912 ascospores were produced in November; in 1914, on the other hand, no ascospores were produced. It is then probably significant that August, September, and October, 1912, had a total precipitation of 3.88, 3.28, and 4.50 inches respectively, as against 2.54, 0.32, and 3.55 inches for the corresponding months in 1914, a difference of over 4 inches for the three months in favor of 1912. This difference is best seen from the graphs, figure 3. Distribution of rainfall is probably more important to the fungus than its total amount since most of the moisture for the growth of the fruiting bodies of the fungus must come from the outside.[6] The three months under consideration had 34 days with more than 0.01 inch of rainfall in 1912 and only 10 in 1914.

Even this difference, however, does not give an adequate idea of the difference in the two years, or of the extent and severity of the drought of September, 1914. In August, 1912, the 3.88 inches of rain came mostly after the middle of the month, the 14 rainy days in September were well distributed and October had a rainfall nearly an

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[6] The number of days with rain is of great importance to all vegetation in such a region as that on Overlook Mountain where the run-off is very great and comparatively little moisture is left in the soil. The writer has discussed the run-off of this region in another connection (12, p. 265).

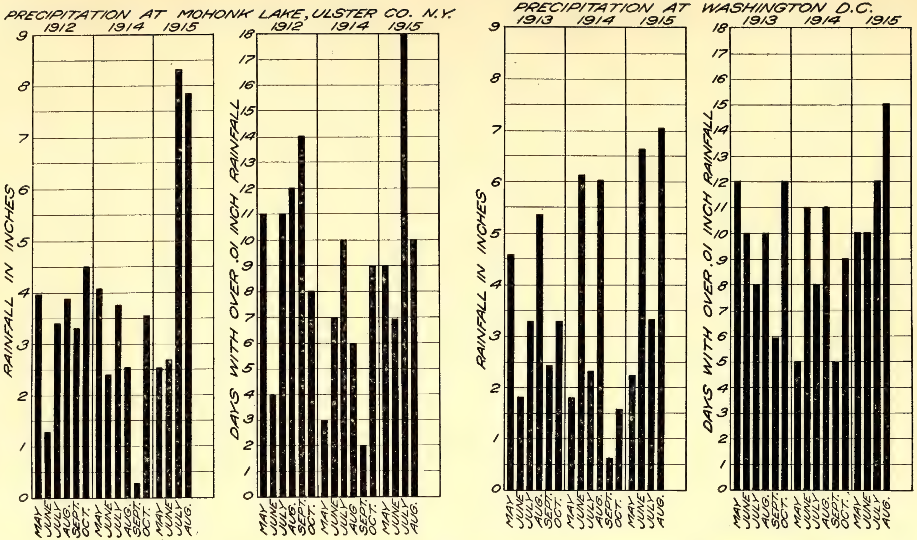

Fig. 3. Graphs showing monthly rainfall and number of days with rain for the growing seasons during three years in Ulster County, New York, and Washington, D. C.

inch above normal. Quite different conditions prevailed in 1914. There was no rain in August after the 21st, only 0.32 inch in September, and no rain in October until the 16th, when two days' rain gave the 3.55 inches of rain recorded. It will be seen then that during almost two months from August 21 to October 16 there were only two days with appreciable rain and these totaled only 0.32 inch, while from August 21 to November 1 there were only four days with any rain. It is of course by no means certain that this extreme drought was the cause of the total failure of the numerous cankers of *Endothia parasitica* to develop perithecia. The condition is, however, very suggestive, and it seems highly probable that a causal relation exists.

Observations near Washington, D. C.

The number of inoculations made near Washington, D. C., is much smaller than of those made in Ulster County, New York. The data available, however, indicate a similar relation between climate and ascospore production. Table XII gives the climatological data for the seasons of 1913, 1914, and 1915 at Washington, D. C. There

Table XII

Monthly Climatological Data for Three Seasons at Washington, D. C.

	Temperature			Precipitation	
	Mean	Summation	Efficiency	In Inches	Days with Over .01 Inch
1913					
May	64.4	602	67	4.55	12
June	73.0	815	91	1.81	10
July	78.0	1011	112	3.24	11
August	74.0	909	97	5.43	10
September	67.0	676	75	2.41	6
October	59.0	417	53	3.37	12
November	48.0	130	25	2.20	8
1914					
May	67.0	717	76	1.72	5
June	73.8	870	94	6.20	11
July	75.9	960	106	2.32	8
August	76.4	892	98	6.00	11
September	66.0	643	72	0.66	5
October	60.0	481	56	1.56	9
November	45.4	139	25	4.49	4
1915					
May	62.5	549	63	2.18	10
June	70.6	774	84	6.58	10
July	76.1	973	105	3.21	12
August	74.0	905	98	7.00	15

is little difference in the temperature of the three summers, although 1913 was somewhat warmer than the others. Both 1913 and 1914, the years in which no perithecia were produced, had a decided drought in the fall months. 1915, on the other hand, when perithecia appeared abundantly by September, had 7 inches of rainfall in August. In this locality, as in Ulster County, New York, perithecia appeared following a period of abundant rainfall and failed to appear in dry weather. It is somewhat surprising that perithecia failed to appear in August, 1914, since the months in Washington had a larger rainfall than the fall months of 1912 in Ulster County. On the other hand, the temperature was much higher in Washington during August, 1914, and the humidity, therefore, presumably lower. This would indicate that it is humidity rather than rainfall as such that determines the production of perithecia. These data are in accord with the assertion originally made by Metcalf (7) that in dry weather spore production was reduced and that dry seasons checked the progress of the chestnut blight.

On comparing the climatological conditions at the two stations for the three years during which observations were made, it is evident that those years in which most ascospores were produced were the years of most abundant rainfall and largest number of days with rain regardless of temperature. If these conclusions are correct, temperature has very little relation to the production of ascospores by *Endothia parasitica*, whereas amount of moisture in the air has a determining relation. This is probable on theoretical grounds since perithecia develop on the dead tissues of the canker separated by a considerable distance from any living tissues of the host, so that moisture which reaches the developing perithecia must necessarily come from the air.

Summary

A quantitative comparison of the available climatic data with the growth and fructification of *Endothia parasitica* at various points from southern New Hampshire to central Virginia has been made.

The area covered includes the northern limits of growth of other species of Endothia and is a transition region for several important plant diseases.

Eleven stations, extending through five degrees of latitude, were chosen, as well as a series of stations at different elevations on Overlook Mountain in the Catskills.

The stations were visited regularly during the summer of 1914.

At each visit ten or more inoculations were made on healthy chestnut trees and notes taken as to the growth of the previous inoculations.

The average annual lateral growth was found to be least at the most northern locality, Concord, N. H., and to increase gradually southward. The growth at Charlottesville, Va., was nearly twice as great as that at Concord, N. H.

A similar relation was found among inoculations made on Overlook Mountain, the amount of growth at elevations of 600 to 1,000 feet being from 20 to 25 percent greater than that at elevations of 2,500 to 2,900 feet.

The stations were all located near regular U. S. Weather Bureau observation stations and no meteorological observations were taken. This necessitated neglecting evaporation entirely, though evaporation is probably less important in the case of a parasitic fungus growing under the bark of a tree than in the case of most green plants.

The difference in the amount of growth of *Endothia parasitica* at the various stations seems to bear no relation to the amount or frequency of rainfall. Amherst, Mass., and Charlottesville, Va. had practically the same rainfall, yet the growth at the latter point averaged nearly eleven cm. greater. On the other hand, stations differing widely in rainfall showed practically the same amount of growth.

The length of frostless season is apparently unimportant, as the fungus has no dormant season. Low temperature retards or even prevents its growth, but growth is resumed as soon as favorable temperature returns. Cultures kept at temperatures as low as 1° C. for twenty-four hours resumed growth almost immediately when removed to room temperature and grew as rapidly as cultures which had never been chilled.

The amount of growth at the various stations is very closely related to the duration and intensity of favorable temperatures.

In tracing the relation between temperature and growth, temperatures were calculated by direct summation as well as by the method of temperature "efficiencies" suggested by Livingston and the results of the two methods compared. The methods give nearly parallel results, though temperature summations agree slightly more closely with amount of growth than do temperature efficiencies.

The time necessary for the development of pycnospores is so short that the climatic factors involved could not be traced.

The fungus in some cases continued to grow parasitically for over eighteen months without producing ascospores.

No mature perithecia were developed at any of the stations during 1914.

Perithecia and ascospores were produced in abundance at many stations during the late winter as well as the spring and summer of 1915.

Air temperature had very little relation to the development of ascospores. They were matured both in midwinter and in midsummer near Washington, D. C., in 1915.

There is a fairly constant relation between the development of ascospores and the amount of atmospheric moisture.

Perithecia were frequently first observed in the spring, a season characterized by high humidity.

The abundant rainfall during the summer of 1915 was accompanied by abundant ascospore production.

The results obtained by Rankin in Ulster County, New York, during the summer of 1912 agree with those obtained by the writer in 1915.

A comparison of the climatological conditions of Ulster County, New York, and Washington, D. C., for three seasons shows that years in which ascospores were produced were the years of most abundant rainfall and largest number of days with rain regardless of temperature.

During the period under investigation dry weather has certainly tended to reduce the spread of the chestnut blight by reducing spore production.

From the data presented in this paper the chestnut blight may be expected to spread somewhat more rapidly in the Southern States than it has in Pennsylvania and the states farther north.

Bureau of Plant Industry,
Washington, D. C.

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Bureau of Plant Industry,
U. S. Department of Agriculture

GUNNERA PETALOIDEA GAUD., A REMARKABLE PLANT OF THE HAWAIIAN ISLANDS

Vaughan MacCaughey

A distinctive feature of the Hawaiian flora is the prevailing endemicity of the rain-forest species. About 85 percent of the flowering plants of the islands are endemic, and the bulk of these are characteristic of the rain-forest zone. This zone lies between the elevations of 2,000–6,000 ft. The mountains of Kauai, Oahu, East Molokai, West Maui, and the Kohala Range on Hawaii, rise to heights of 3,000–6,000 ft., and thus their summits are covered with dense rain-forest vegetation. The great valleys of erosion have eaten back into the very hearts of these mountain masses, so that the summit regions abound in knife-edged ridges and great precipices. Many of the summit ridges are only three or four feet wide at the crest; many of the precipices are 800–1,800 ft. high. The rainfall in these regions is torrential, and much of the vegetation is of the most pronounced hygrophytic type.

One of the most characteristic and conspicuous plants of these humid summit regions is the endemic halorrhagaceous *Gunnera petaloidea* Gaud.[1] In the little hanging valleys that abound in this zone, on the precipices as well as in the steep stream-beds, are masses of this titanic herbaceous-perennial. The gigantic leaf-blades are three to four feet in diameter, peltate on fleshy petioles two or more feet long.[2] The petioles arise from a creeping or erect rhizome, which is fleshy, green, and four or five inches in diameter. The huge crown of leaves springs from the apex of the rhizome. As the latter is often branched, the total mass of foliage was spread over an area of ten or twenty square feet, with a height of six or eight feet. In places where they have not been disturbed by the landslides that are common in these regions, these gigantic herbs often cover areas fifty to a hundred feet long and twenty or more feet wide, as on the upper slopes of a precipice, where they form a beautiful mural tapestry.

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[1] See bibliography.

[2] The blades of the Chilean *G. manicata*, the largest of the genus, are 5–10 ft. in diam., on petioles of 6–7 ft.; these are used in Chile for tanning hides.

The rhizome is very soft, and can be severed by a single machete stroke. It contains a considerable quantity of crude starch, together with numerous conspicuous fibers. It frequently contains colonies of endophytic algæ. It is closely pressed to the wet soil, but is not subterranean; it roots freely along the entire undersurface. The older, naked portions of the rhizome are green and conspicuously marked with the large petiole scars. No bark is developed. The apical region, 18–24 inches long, is usually more or less erect, depending upon the situation; sometimes, as near a stream-bed, the rhizome stands erect to a height of three or four feet. The entire length of the rhizome is generally not over six or eight feet; its frequent branching and the decay of the older parts tend to separate an old rhizome into several shorter new individuals. This vegetative reproduction, quite similar to that of many ferns, is the common mode of propagation after the plant has once established itself.

The petioles are thick, fleshy, and curiously muricate; they are three to four inches in diameter, and two to four feet long. The broad, fleshy stipules, 1–1½ in. long, are adnate to the base of the petiole. The blade is orbicular or rounded-reniform. It stands at right angles to the petiole. Its attachment is peltate, but there is a broad, open, basal sinus. It is very thick and fleshy, and deeply rugose. Gray states that the blades are "1½–2 ft. in diam. when full grown"; Hillebrand, that they are "2–3 ft. in width"; both of these are underestimates, and evidently based upon the examination of herbarium material, rather than a knowledge of the plant in the field. Leaves that are fully expanded are commonly three to four feet in diameter, and Bryan records a diameter of five feet.

The blade is more or less conspicuously eight- to ten-lobed, the lobes being very shallow, rounded and coarsely dentate. On its upper surface the blade is covered with coarse, short hairs; the under surface has a strong network of prominent veins. There are five large veins, pedately arranged; the venation is dichotomous, and more or less hispid with short, coarse hairs. A variety *beta*, collected by the U. S. Exploring Expedition on Kauai, and described by Asa Gray, has nearly glabrous foliage, with "bracts ovate or oblong, 6–8 mm. long."

The main flowering season is mid-summer, although there seems to be considerable variation. The panicles are terminal. The rachis is 2–3 ft. tall, hirsute and scabrous, branching from near the base, and grooved. The branches or spikes are 4–9 inches long, undivided, crowded but lax and spreading; they are covered throughout with clustered or scattering sessile flowers. The bracts of the inflorescence are linear, scarious, 1–1½ in. long. The flowers are bisexual and not bracteolate. The calyx is globular with adnate tube; there are two lobes, one anterior and one posterior; these are persistent, each 1–2 mm. long, broadly ovate or triangular, with broad or truncate apex, denticulate or 3-toothed, with a raised line along the inner face. The petals are two, alternate with the calycine lobes and 2 or 3 times as long; cucullate, enclosing the stamens before anthesis; broadly ovate or cuneate, retuse, obscurely glandular on the back, thickish in texture, epigynous; tardily deciduous. The stamens are two, epigynous, opposite the petals; filaments very short; anthers large, about 2 mm. long, emarginate at each end, somewhat didymous, fixed by the base, introrse, the two cells opening longitudinally. Pollen grains four-lobed. Styles two, opposite the stamens, and nearly twice their length; linear-subulate, hispid, slightly united at the base. Ovary one-loculed, with a single anatropous ovule suspended from the summit of the locule. Drupes ovoid-globose, yellow, reddish or purple, 2–4 mm. long, crowned with the short and incurved calycine lobes; the calyx tube forming the fleshy sarcocarp; endocarp small in proportion; acheniform, lenticular, 3- or 4-angled, crustaceous. Seed conformed to the endocarp; testa very thin and delicate; embryo minute, near the hilar extremity of the fleshy and oily albumen, subcordate, the radicle superior.

Schindler's monograph of the Halorrhagaceae in Engler's Pflanzenreich contains the following detailed description of *petaloidea:*

> "Statura maxima, metrali vel ultra; rhizomate crasso, haud stolonifera, folia subpauca rosulata apice procreante, ligulis chartaceis, glabris, ± 45 mm. longis, latissime ellipticis apice obtusis, pluries divisus induto, scapos floriferos complures axillares proferente.
>
> "Folia maxima, petiolo validissimo, ± 0.6 m. longo, canaliculato, basi laxe piloso superne glabro vel glabrato, hinc inde aculeolis brevibus sueto fere punctiformibus instructo stipitata; lamina depresse reniformi sat latiore ac longa, latissime cordata, circuitu in lobos subaequales sueto 9 late rotundatos vel obtusos divisa, margine grosse crenata, dentibus junioribus apice apiculatis senioribus obtusis, supra plana nec prominenti-areolata, praeter nervos nervillosque supra perlaxe subtus densius pilis crassis conspersos glabra, usque as 0.5 m. lata mihi visi.
>
> "Inflorescentia scapo crasso, lineatim angulato, arcuatim adscendente, brevi sed semper manifesto, juniore saltem dense pilis crassis conicis consperso elata, quam folia bervior, ∞ flora, densior laxiorve, optime thyrsoidea, apice breviter acuta,

± 0.4 m. longa, axi primario crasso, piloso, bracteis primariis conspicuis quidem sed tamen quam ramuli axillares multo brevioribus, linearibus, apice subrotundatis, glabris, integerrimis vel basin versus obscure denticulatis, 15 mm. vix excedentibus, ramulis basi brevissime sterilibus, suberectis, pilosulis, primum dense demum in florum statu ♀ elongatis laxius quaquaverse flores ∞ gerentibus, usque ad 150 mm. longis mihi visis sueto brevioribus; flores sessiles, 5 mm. longi, glaberrimi; ovarium laeve, breviter lateque cylindricum, apice vix constrictum, minute 4-lineatum; sepala brevissima, late squamiformi-triangularia, apice acuminulata, 0.5 mm. longa; petala glabra, ex ungue brevi late lineari in laminam haud multo latiorem, cucullatam, apice obtusam producta, ± 2 mm. longa; stamina quam petala sat breviora, crassa, antheris fere orbicularis, apice obtusis, laevibus, quam filamenta brevissima crassaque longioribus; styli crasse cylindrici apice acuti, dense papilloso-villosi.

"Bacca exsucca, globosa, laevis, ± 2.5 mm. diam. metiens."

The family Halorrhagaceae Schindler[3] comprises seven genera. The family includes aquatic and terrestrial perennial herbs of widely diverse habit; some are minute, others, like the Hawaiian species, are titanic in size. The flowers are small, axillary or in terminal racemes or panicles, bi- or uni-sexual, regular; sepals usually 4, petals usually 4 or 0; stamens 8, the outer opposite the petals, or 4, rarely fewer; ovary inferior, 1–4-loculed, each locule one-ovuled; fruit nut-like, often crowned by the calyx.

The representation and geographic distribution of the genera is as follows:

1. *Loudonia* Lindl.—3 species; Australia.
2. *Halorrhagis* Forst.—about 60 species; Australia, Tasmania, New Zealand, Chatham I., New Caledonia, Chile, Juan Fernandez, China, Lower India.
3. *Meziella* Schindler—1 species; Australia, aquatic.
4. *Laurenbergia* Berg.—18 species; Africa, Mauritius, Bourbon, Ceylon, East Indies, Java.
5. *Proserpinaca* L.—2 species; "Mermaid Weed"; North America, Canada to Guatemala, in standing and slow-running water.
6. *Myriophyllum* L.—36 species; "Parrot's Feather"; cosmopolitan, all continents, including Australia and many islands.
7. *Gunnera* L.

The last genus, Gunnera, was named in honor of Ernst Gunner, a Swedish bishop and botanist (1718–1773), who wrote a local flora. In Gunnera the leaves are radical, ovate or orbicular, and often gigantic. The flowers are perfect, or rarely imperfect monoecious or polygamous; small, greenish, in simple or branched spikes or panicles, the staminate flowers on the upper branches; flowers often packed on a great cob-like spike; petals 2 or 3 or none; calyx none or with 2 or 3 lobes; stamens 1, 2, or 3; ovary 1-loculed, bearing 2 filiform styles; fruit a drupe; plants rhizomatous.

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[3] Britton and Brown use the spelling Haloragidaceae, and include the genus Hippuris, making eight genera.

The geographical distribution of the known Gunnera species is as follows:

Sub-gen. I. Milligania (Hook. f. emend.) Schind.

1. *cordifolia* Hook. f.; Tasmania.
2. *monoica;* New Zealand, Chatham.
3. *mixta* Kirk; New Zealand.
4. *strigosa* Colenso; New Zealand.
5. *prorepens* Hook. f.; New Zealand.
6. *densiflora* Hook. f.; New Zealand.
7. *dentata* Kirk; New Zealand.
8. *arenaria* Cheeseman; New Zealand.
9. *hamiltonii* Kirk; New Zealand.

Sub-gen. II. Misandra (Comm.) Schind.

10. *lobata* Hook. f.; extreme S. America.
11. *magellanica* Lam.; high mountains of S. Amer., Colombian Andes, Chile, Patagonia, Ecuador, etc.; alpine.
12. *reichei* Schind.; Chile (1,800 meters elev.).

Sub-gen. III. Pseudo-Gunnera (Oerst.) Schind.

13. *macrophylla* Blume; New Guinea, Celebes, Java, Sumatra, Philippines, in high mountains.
14. *perpensa* L.; S. and E. Africa, Madagascar.

Sub-gen. IV. Panke (Mol.) Schind.

15. *petaloidea* Gaud.; Hawaiian Islands only.
16. *bracteata* Steud.; Chile, Juan Fernandez.
17. *glabra* Phil.; Chile, Juan Fernandez.
18. *pyramidalis* Schind.; Chile, Juan Fernandez.
19. *peltata* Phil.; Chile, Juan Fernandez.
20. *pilosa* Kunth.; Colombia and Ecuador, high mountains.
21. *boliviana* Morong; Bolivia.
22. *apiculata* Schind.; Bolivia, high mountains.

23. *rheifolia* Schind.; Peru.
24. *brephogea* Linden; Colombia and Ecuador.
25. *manicata* Linden; Colombia.
26. *berteroi;* Phil.; Chile, high mountains.
27. *chilensis* Lam.; Chile, high mountains.
28. *brasiliensis* Schind.; Brazil.
29. *vestita* Schind.; Chile.
30. *commutata* Blume; Chile.
31. *insignis* (Oerst.) DC.; Costa Rica.
32. *wendlandii* Reinke; Costa Rica.
33. *insularis* Phil.; Juan Fernandez.

It is extremely significant to note that *G. petaloidea* is one of a number of endemic Hawaiian plants that have very close affinities with the Andean flora. It has been suggested that at one time in the history of the Pacific there existed a land-bridge or its equivalent connecting the now-remote Hawaiian archipelago with the South American continent. Considerable evidence could be brought forth to substantiate this view.[4]

Some of the typical habitats of this remarkable herb are: Wai-ale-ale Swamps, Kauai (4,000–5,000 ft.); Ka-ala and Kona-hua-nui summit ridges on Oahu (2,500–4,000 ft.); Pele-kunu Pali, Molokai (3,000 ft.) East and West Maui mountains (3,000–5,000 ft.); and the Ko-hala Range of Hawaii (4,000–5,000 ft.). It is thus evident that the Hawaiian Gunnera occupies a distinct ecological zone—2,500–5,000 ft.—which in general is characterized by steep declivities and torrential precipitation. It is never known to occur above or below the limits of this zone, although its drupes could be easily carried by birds, and it has abundant opportunity to descend mechanically to the lower levels. A striking peculiarity for a plant of such magnitude is its *strong "preference"* for very steep slopes, upon which it maintains an apparently precarious footing. These slopes have the advantage of maximum illumination, but are constantly subjected to landslides. In many of the regions enumerated above, Gunnera forms a tapestry on inaccessible and nearly vertical cliffs. Field studies of Gunnera give the impression that it has attained a relatively static condition, with reference to range, and is neither markedly spreading nor losing ground.

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[4] The ecology of Gunnera indicates that it has been a member of the Hawaiian flora for a very long period of time; it belongs to the primitive flora.

The Hawaiian name for this plant is *Apé* or *Apé-Apé;* so far as is known the natives did not utilize this plant in any way. Some of the Gunneras of other regions are used horticulturally to produce luxuriant foliage effects, for which purpose they are admirably adapted. The Hawaiian species has not been utilized in this way; it is associated only with the fog-swept precipices of Hawaii's beautiful rain-forests.

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College of Hawaii,
Honolulu, Hawaii

AN INTERESTING MODIFICATION IN XANTHIUM

CHARLES A. SHULL

Two years ago I received through the kindness of Mr. F. F. Crevecoeur, of Onaga, Kansas, some burs of Xanthium which show a very unique and interesting modification. The ordinary burs of Xanthium are too common and familiar to need description. Normally they enclose but two ovaries, and possess only two beaks which arise conjointly from the outer end of the bur. Through these beaks the styles protrude at the time of pollination.

These modified burs, however, enclose a considerable number of ovaries, usually between twenty and thirty. The beaks are correspondingly increased in number, and are arranged in two or three concentric rows about a central depression which occupies the central part of the distal half of the bur. Figure 1 shows the burs about natural size. The form of the bur is probably determined by the cessation of growth by the centripetal portion of the receptacle, while the centrifugal zone continues to develop, and imbeds a number of flowers which are apparently arranged in more or less concentric rows. Figure 2 shows one of the burs with the outer wall of the receptacle removed so as to show the outer row of seeds, each of which is enclosed in its black ovarial wall.

The exact structure of the bur is most easily understood from an examination of the cross section of the bur taken slightly above the equator, just beyond the bottom of the depression previously mentioned. Such a section is shown in Figure 3. Not all of the burs had the same number of ovaries, but the general structure of all was the same. In this particular bur there were twenty-six ovarial cavities in the receptacle, twenty-three of which contained the remains of ovaries. The position of the cavities which contained ovaries are indicated by small circles. The other three cavities contained no trace of ovaries, but their position indicates clearly enough that they correspond to a third row of florets.

There is a very strong tendency to sterility, apparently, for many of the ovaries were empty. Of the twenty-three ovaries found in the bur shown in Figure 3, only twelve contained seeds. And a small box of burs coming from direct descendants but two generations removed from the original plant showed complete sterility, entire absence of seeds.

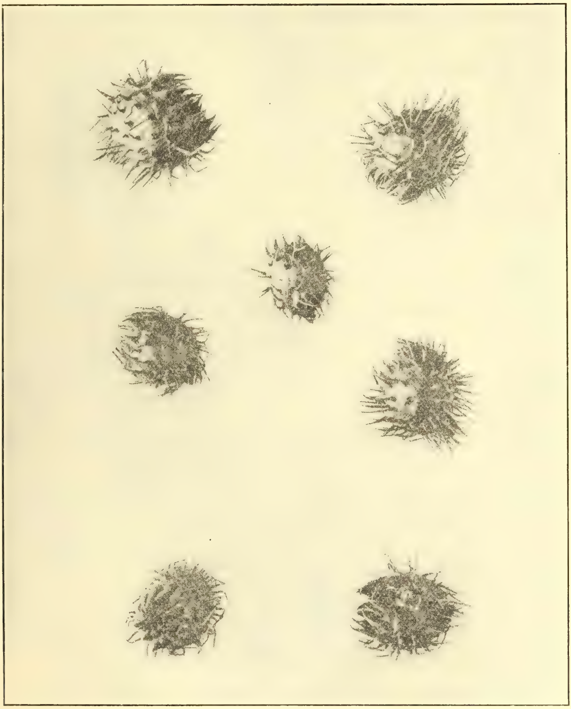

FIG. 1. *Xanthium canadense* var. *globuliforme* Crevecoeur. Burs about natural size.

The history of this interesting race of cockleburs extends through three generations, beginning in 1909, at which time the original parent was discovered growing in a corn field, by a farmer living near Onaga, Kansas. This parent plant was given to Mr. Crevecoeur, who planted some of the burs in his flower garden in the spring of 1910. He secured a number of plants from them, and reports that "a portion of the plants bore the same kind of burs as the well known *X. canadense*, while the major portion bore the same kind as the seed planted." This would seem to fix the relationship of the type to *X. canadense*, and Mr. Crevecoeur labelled the specimen in his herbarium *X. canadense* var. *globuliforme*.

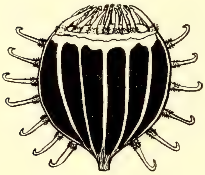

FIG. 2. Bur with wall removed to show seeds.

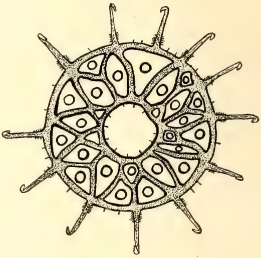

FIG. 3. Diagrammatic cross section of a bur showing arrangement of seeds.

The following year, 1911, seeds of the plants grown in 1910 were sent to Miss Grace Meeker, of Ottawa, Kas., who secured a third generation. All of the plants in this third generation were of the *globuliforme* type, but they produced burs which were small, and devoid of seeds. With these plants the group became extinct.

Mr. Crevecoeur sent me quite a number of burs, and many seeds were planted, but all were non-viable. In some cases patches of cells in the cotyledons were still alive, but not a single hypocotyl showed signs of life. The early loss of viability in this case was partly due, no doubt, to the fact that the plants of the second generation, 1910, were destroyed while the burs were somewhat immature, in order to prevent possible escape from cultivation. It is quite natural that one sholud not desire the survival of a cocklebur producing twenty or more seeds to the bur!

In the spring of 1915 two of the burs still remaining from the original parent were planted at Onaga, but the seeds did not germinate. The possibility of studying the inheritance of the bur characters in crosses and self-fertilized strains is thus precluded so far as this local appearance of the variety is concerned. From two other sources have come vague reports of the same varieties in other localities, but investigation has failed to uncover them. However, it is possible that intelligent observation by field botanists might lead to their rediscovery.

Nothing is known regarding the cause, or manner of origin, of the *globuliforme* type. The character of the modification is such that it could hardly result from hybridization, although splitting was noted in the 1910 generation. The cause of the sterility is merely conjectural and might be due to various factors. Sterility of pollen, if it really occurs, would not necessarily indicate a case of hybridization.

It seems more reasonable to consider it a mutation from *X. canadense* Miller. Whether it is progressive, a new condition, or retrogressing toward remote ancestry, one cannot tell. But in view of Farr's recent studies on the origin of inflorescences and dicliny in Xanthium,[1] the latter possibility is particularly significant. Farr reaches the conclusion that the bur is a reduced capitulum, in which the florets now, of course, are reduced to two. If this *globuliforme* type is a reversional mutation, it gives a concrete idea of the kind of capitulum from which the reduction has occurred. Such a concrete picture is a distinct advantage in any attempt to depict the lines along which such an evolutionary advance has proceeded.

THE UNIVERSITY OF KANSAS,
LAWRENCE, KANSAS

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[1] Farr, Clifford H., The Origin of the Inflorescences of Xanthium. Bot. Gaz. **59**: 136–148. 1915.

ENDOPHYLLUM-LIKE RUSTS OF PORTO RICO

E. W. Olive and H. H. Whetzel

The writers recently spent a little over two months in Porto Rico, from February 23 to April 26, 1916, collecting and studying mainly the parasitic fungi. A fairly representative lot of rusts were collected from many localities about the Island. Among these were five aecidioid and one peculiar uredinoid form which, after germination studies, we found to be short-cycled and similar to, if not indeed identical with, the Endophyllums.

We wish to acknowledge special obligation to Professor J. C. Arthur, not only for determining all our rust collections after our return, and for making many suggestions in the preparation of the systematic portion of this paper, but also for directing our attention, prior to our journey, to certain unsolved problems, in particular to the urgent need of clues in the case of the unconnected aecidia of Porto Rico. For the preparation of the agar-water medium and for many other courtesies we are much indebted to Plant Pathologist E. W. Brandes and Director May of the Federal Experiment Station, as well as to Dean Garwood, Professor C. E. Hunn and others of the Agricultural College at Mayagüez. For laboratory facilities and for other freely tendered assistance we are also under great obligations to Mr. J. A. Stevenson, plant pathologist, and to Director Tower, of the Insular Experiment Station at Rio Piedras. After our return from Porto Rico, most of the hosts of our fungi were determined by Director Britton and others of the New York Botanical Garden; the grass hosts by Professor Hitchcock and Mrs. Chase; the ferns by Miss Slosson, to all of whom we desire to acknowledge our great indebtedness. We wish to express our thanks especially to Mr. Percy Wilson of the staff of the New York Botanical Garden, who for several days so generously placed his wide knowledge of West Indian plants entirely at our disposal.

Arthur's "Uredinales of Porto Rico, based on collections by F. L. Stevens,"[1] which proved so very stimulating in our search, enumerates 10 aecidium-forms, all of which he at that time supposed to be heteroecious. The discoveries of Kunkel[2] in the case of *Caeoma nitens* (Schw.) Burrill and of Fromme,[3] in connection with *Aecidium tuberculatum* Ellis and Kellerm., by means of which they proved the teliosporic character of the supposed aecidiospores, also acted as a great stimulus in our work. We tried to a limited extent the agar medium recommended by Kunkel, but, laboring under the rather trying tropical conditions, we came finally to use almost exclusively the water surface method. This method proved very efficient, as well as very simple and easy to manipulate. All our germinations were tested successfully again and again by sowing the spores on the surface of water drops placed on slides which were supported up from the bottom of moist chambers. Inverted Petri dishes, with a little water in the bottom to seal the cover, served admirably for the latter.

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[1] Mycologia **7**: 168–196, 227–255, 315–332. 1915; **8**: 16–33. 1916.

In order to secure the best results, the spores must float on the surface of the water, so that their germ-tubes may grow up into the moist air. If, on the other hand, the spores are completely immersed, the tubes then appear much like those from true aeciospores. We found also that by chopping up bits of the host tissue with the sori on them, and putting these so that they were not covered with water but merely wet, much better and more abundant germination of the telia resulted.

When once we became convinced of the short-cycled character of one of these aecidium-like rusts, we became suspicious of all and determined to try out the spore germinations of every aecidioid rust with which we came in contact. Our first successful find was in connection with *Aecidium Wedeliae*, one of the commonest and most widely distributed of Porto Rican rusts. Professor Arthur states[4] that Dr. Stevens had made the suggestion that the alternate host in this case might be Cyperus, bearing *Puccinia canaliculata* (Schw.) Lagerh. However, the very commonness of the Aecidium, occurring as it does in all sorts of situations, all over the Island, on the host *Wedelia trilobata*, combined with the comparative rarity of the Cyperus hosts bearing *Puccinia canaliculata*, made us at once doubtful as to any possible connection between the two. As stated above, our suspicions were confirmed when trials of the germination of *Aecidium Wedeliae* showed that the spores produce at once promycelia and that this form is therefore a short-cycled Endophyllum and not a heteroecious form, as had been thought.

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[2] Bull. Torrey Bot. Club **40**: 361–366. 1913; **43**: 559–569. 1916. Amer. Journ. Botany **1**: 34–37. 1914.

[3] Bull. Torrey Bot. Club **42**: 55–61. 1915.

[4] *L. c.*, p. 318.

In all, we germinated 13 aecidioid and uredinoid forms, in some cases repeating the experiment several times in order to confirm our earlier observations. In 7 of these, the spores germinated very sparsely and very slowly; resulting at the end of 24–48 hours in a few long, unseptated germ-tubes. We therefore became convinced that in these 7 species (*Aecidium passifloriicola* P. Henn., *A. tubulosum* Pat. & Gaill., *A. Tournefortiae* P. Henn., *A. abscedens* Arth., *A. Borreriae* Pat., *Uredo Trichiliae* Arth. (ined.), and the aecial stage of *Uromyces proëminens* (DC.) Pass.) we were dealing in all probability with true aecia and therefore with long-cycled forms. We secured, in fact, considerable evidence in two of the above cases as to possible alternate hosts; coming to the conclusion that the first species was probably associated with *Puccinia Scleriae* (Paz.) Arthur and the second with *Puccinia substriata* Ellis & Barth. Mr. Stevenson, of the Experiment Station at Rio Piedras, had also come to a similar conclusion in the case of the second—*A. tubulosum* on Solanum.

The slow and meager germination of the true aeciospores of the above 7 forms is in marked contrast to that of the spores of the short-cycled rusts described below. In the latter case, in an incredibly short time, 10 or 12 hours or even less, nearly all of the spores germinated. When these spores are floated on the surface of water drops in moist chambers, they push out into the free air a profuse mass of unbranched, septate promycelia (basidia), each bearing the 4 (or in some cases only 2) basidiospores (sporidia). It must be kept in mind, however, in germinating these forms, that a source of error is liable to arise if one is not extremely careful in the floating of the spores. When entirely immersed, they always grow out into long tubes, rarely forming sporidia, and might thus easily be mistaken for ordinary aeciospores.

The germ-tubes vary considerably in length as well as in other characteristics in these Endophyllum-like forms. Sometimes, indeed, even in the same lot of germinations, there is considerable variation, due perhaps to their being grown sometimes in moist air only, sometimes partially in water. Two of the species showed, however, a most remarkable variation, which is, in contrast to the above, apparently not at all environmental. The spores of *Endophyllum Stachytarphetae* and of *E. circumscriptum*, on germinating, produce only 2 spores to each promycelium, instead of the normal 4 basidiospores. We are as yet uncertain as to the constancy of this character; neither are we yet oriented as to its probable significance.

The general characters and systematic arrangement of the short-cycled rusts which we have found to produce promycelia are described below, under 6 species. These are all considered in our title to be Endophyllum-like, although it will be noted that only 4 of the 6 species are really placed in this genus. The first one described is, in fact, not at all aecidioid, but uredinoid in its fructifications; while the last one of the list, while aecidioid, differs sufficiently from Endophyllum to justify its being placed in a separate genus.

Botryorhiza Whetzel & Olive, gen. nov.

Cycle of development includes only telia.

Pycnia unknown (probably not formed).

Telia subepidermal, erumpent; teliospores thin-walled, oval, one-celled, borne singly on long pedicels; each germinating apically on maturity to produce a promycelium with 4 basidiospores; haustoria botryose, or irregularly branched.

Type species, *Botryorhiza Hippocrateae* Whetzel & Olive, on *Hippocratea volubilis*. The generic name is derived from the fact that this form produces large, botryose haustoria, a character apparently occurring also in certain smuts.[5]

Botryorhiza Hippocrateae Whetzel & Olive, sp. nov.

O. Pycnia wanting (probably not formed).

III. Telia mostly hypophyllous but sometimes amphigenous or caulicolous, generally from a localized mycelium, sometimes from a systemic invasion affecting entire young shoots; localized sori densely crowded in more or less orbicular or irregularly shaped, somewhat hypertrophied pulvinate areas, 1 mm.–1 cm. or more across, the affected areas yellowish when young, when older becoming whitish due to the germination of the spores; in older leaves often killing affected spots, which turn brown, the resultant rounded, swollen dead areas then bearing a striking resemblance to certain insect galls.

Telia pulverulent, erumpent, from a definite, superficial, uredinoid hymenium which arises just under the epidermis, without peridium; teliospores uninucleate, borne singly at the end of pedicels which arise from a binucleate mycelium, 13–14 by 18–24 μ, thin-walled, oval, with a rounded apical protuberance, germinating apically at maturity to produce each a long, cross-septate basidium (promycelium) bearing 4 basidiospores (sporidia), similar in shape to the teliospores and 8 by 11–12 μ.

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[5] Lutman (Some contributions to the life history and cytology of the smuts. Trans. Wis. Acad. Sci. **16**: 1191–1244. 1910) has figured botryose haustoria in *Doassansia deformans*. (See his figs. 44, 45.)

Vegetative mycelium composed of coarse intercellular hyphae, made up of binucleate cells, some of which send large botryose, or irregularly shaped, haustoria into adjacent cells.

On Hippocrateaceae:

Hippocratea volubilis L., Porto Rico (W. & O. No. 87, *type*; *figs*. 1, 2).

It would indeed be peculiar if this conspicuous fungus had entirely escaped description. We are, however, unable to find any published matter pertaining to it. It is, perhaps, not so strange that it has escaped inclusion in the rusts. In the collections at the Agricultural Experiment Station at Rio Piedras we found it classed as an insect gall; really quite a logical place for an old specimen, when judged alone from its gross appearance.

As is well known, many tropical rusts are pale and inconspicuous and otherwise quite unlike the yellowish or brownish rusts with which we are familiar in colder climates; further, according to Professor Arthur, "all of the so-called species of Eriosporangium and Argomyces are white-spored, as well as the uredinia of Uredinopsis and many others." And he adds: "I see no reason why this is not a true rust, although a very unusual one."[6]

It is, indeed, quite likely that the coarse mycelial hyphae and the remarkable botryose haustoria will prove to be unusual features among rusts; and that these are characters which are doubtless more prevalent among smuts than among rusts. But, on the other hand, the spores are cut off externally, much as in Uromyces, from the ends of protruding hyphae; and, further, the spore-bearing hyphae are always produced in a more or less regular, superficial *hymenial layer*, which arises in hypodermal regions, generally just under the epidermis. The latter are undoubtedly rust characteristics and not those of smuts. It is of considerable interest, indeed, to find in this form characters common to both smuts and rusts, thus adding emphasis to the general belief in a common ancestry and a present near relationship for these two great groups.

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[6] In letter of October 6, 1916.

Endophyllum circumscriptum (Schw.) Whetzel & Olive, comb. nov.

Aecidium circumscriptum Schw.; Berk. & Curtis, Journ. Phila. Acad. Nat. Sci. II. **2**: 283. 1853.

Aecidium Cissi Wint. Hedwigia **23**: 168. 1884.

O. Pycnia epiphyllous, few, subepidermal, rarely breaking through the epidermis, about 80–85 μ broad in section.

III. Telia amphigenous but mainly hypophyllous, aecidioid, numerous, crowded, cup-shaped, borne in rounded, somewhat hypertrophied, pulvinate areas; peridium recurved, slit into a few coarse segments; teliospores catenulate, more or less rounded-angular or irregular from pressure, 12–13 by 15–18 μ.

ON VITACEAE:

Cissus sicyoides L., Brazil, Costa Rica, Cuba, Dutch Guiana, Jamaica, Porto Rico, St. Thomas (*figs.* 3, 4).

Endophyllum Wedeliae (Earle) Whetzel & Olive, comb. nov.

Aecidium Wedeliae Earle, Muhlenbergia **1**: 16. 1901.

O. Pycnia probably not formed.

III. Telia mainly hypophyllous, aecidioid, densely clustered, borne in light yellowish areas of somewhat irregular shape; peridia scarcely emergent, evanescent; teliospores catenulate, globoid or more or less angular from pressure, 12–13 by 16–18 μ.

ON COMPOSITAE:

Wedelia trilobata (L.) Hitch. Porto Rico, Jamaica and other West Indian Islands (*figs.* 13, 14).

This is perhaps the commonest of the Endophyllums growing in Porto Rico. As stated above, it was this very abundance that made us suspicious of any possible connection with *Puccinia canaliculata,* as had been suggested by Stevens.

Endophyllum decoloratum (Schw.) Whetzel & Olive, comb. nov.

Aecidium decoloratum Schw. Berk. & Curtis, Journ. Phila. Acad. Nat. Sci. II. **2**: 283. 1853.

Aecidium Clibadii Syd. Ann. Myc. **1**: 333. 1903.

O. Pycnia probably not formed.

III. Telia hypophyllous, aecidioid, in rounded or sometimes irregular, more or less numerous areas, 2–7 mm. in diameter; peridia evanescent, sometimes short cylindrical, with incised margin; teliospores catenulate, globoid or more or less angular from pressure, 12–13 by 16–18 μ.

ON COMPOSITAE:

Clibadium arboreum J. D. Smith, Mexico.
Clibadium Donnell-Smithii Coult., Guatemala.
Clibadium erosum (Sw.) DC., Porto Rico (*figs*. 11, 12).
Clibadium surinamense L. Dutch and French Guiana.

We found this Endophyllum only on the slopes of the eastern mountains of Porto Rico, especially the foothills of El Yunque and El Duque.

Endophyllum Stachytarphetae (Henn.) Whetzel & Olive, comb. nov.

Aecidium Stachytarphetae P. Henn. Hedwigia Beibl. **38**: 71. 1899.

O. Pycnia probably not formed.

III. Telia hypophyllous, aecidioid, one to few in number to each leaf, occurring in rounded, or somewhat irregular, rather inconspicuous, pulvinate areas; peridia evanescent; teliospores catenulate, globoid or more or less angular from pressure, 14–15 by 15–25 μ.

ON VERBENACEAE:

Stachytarpheta cayennensis (L. C. Rich.) Vahl (*Valerianodes cayennensis* (L. C. Rich.) Kuntze) Porto Rico, Santo Domingo, Bolivia, Colombia (*figs*. 5, 6).

Stachytarpheta dichotoma Vahl, Brazil (E. Ule No. 2163.)

According to Professor Arthur, this is the first time this rust has been reported from North America. We found it only at Rio Piedras, in a little valley near the Experiment Station. This, also, was the only locality in which we found the host.

Endophylloides Whetzel & Olive, gen. nov.

Cycle of development includes, so far as is known, only telia.

Pycnia unknown, (probably not formed).

Telia erumpent, the chains of spores adhering to form more or less extended, cylindrical columns, about 2–4 times as long as broad, waxy or horny when dry. Peridium wanting, or at least inconspicuous.

Teliospores catenulate, one-celled, germinating at the apex of the column.

Type species, *Endophylloides portoricensis*, on *Mikania cordifolia*.

This form differs markedly from Endophyllum in that the latter is much more aecidium-like, with usually prominent peridium-cup and pulverulent masses of spores. Similarly, while undoubtedly resembling in some respects the type genus of Dietelia, *D. verruciformis* P. Henn., yet we regard the absence of an evident peridium and the possession of comparatively long, horny columns of teliospores in Endophylloides, in contrast to the strongly developed peridial cells and the globose or subglobose telia in Dietelia, as sufficiently distinctive to warrant the formation of the new genus.

Endophylloides portoricensis Whetzel & Olive, sp. nov.

Aecidium expansum Arth. Mycol. 7: 317. 1915 (not *A. expansum* Diet.).

O. Pycnia probably not formed.

III. Telia chiefly hypophyllous, sometimes petiicolous or caulicolous, short-cylindrical, forming more or less waxy or horny columns about ⅓ mm. in diameter by 0.5–1 mm. long, aecidioid, borne in irregularly shaped areas, 0.5–1 or more cm. in diameter; peridial cells inconspicuous, often collapsed, scarcely forming a continuous peridium; teliospores rounded or oval, 12–15 by 15–20 μ, in long persistent chains, separated from each other by prominent intercalary cells.

ON COMPOSITAE:

Mikania cordifolia (L. f.) Willd., Porto Rico (Whetzel & Olive, No. 83, *type*, *figs.* 7–10).

Mikania odoratissima Urban, Porto Rico.

The first host is found very commonly all over the Island; and the fungus is also quite generally distributed. *Mikania odoratissima*, on the other hand, is, in our experience, much rarer. Our collections of the latter were made only on the mountain slopes of El Yunque and El Duque, at the extreme eastern end of the Island.

BROOKLYN BOTANIC GARDEN AND CORNELL UNIVERSITY

EXPLANATION OF PLATES I–III.

All photos except Fig. 3 were taken by Mr. L. Buhle, of the Brooklyn Botanic Garden. Figure 3 is from a photograph taken in Porto Rico by Prof. Whetzel.

FIG. 1. *Botryorhiza Hippocrateae*, on leaves of *Hippocratea volubilis.*

FIG. 2. The same, enlarged; × about 4.

FIG. 3. *Endophyllum circumscriptum*, on leaf of *Cissus sicyoides;* × about 2.

FIG. 4. The same; × about ⅔.

FIG. 5. *Endophyllum Stachytarphetae*, on leaves of *Valerianodes cayennensis.*

FIG. 6. The same, enlarged; × about 4.

FIG. 7. *Endophylloides portoricensis*, on petiole of leaf of *Mikania odoratissima;* enlarged; × about 4.

FIG. 8. The same; × about ⅔.

FIG. 9. The same, on leaf of *Mikania cordifolia.*

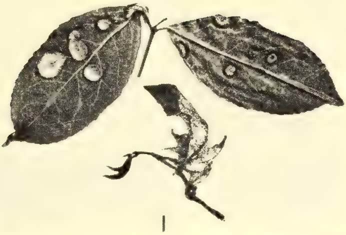
FIG. 10. The same; × about 3.

FIG. 11. *Endophyllum decoloratum* on leaf of *Clibadium erosum;* × about ½.

FIG. 12. The same; × about 3.

FIG. 13. *Endophyllum Wedeliae*, on leaves of *Wedelia trilobata;* × about ½.

FIG. 14. The same; × about 3.



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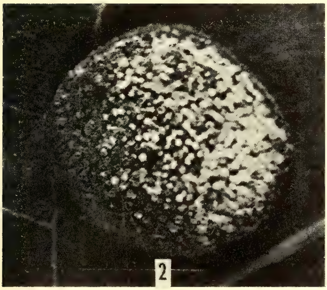

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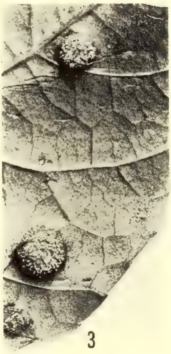

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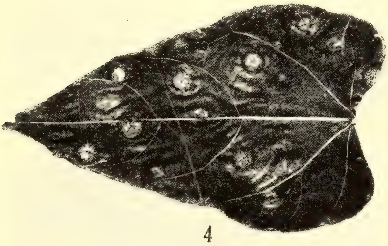

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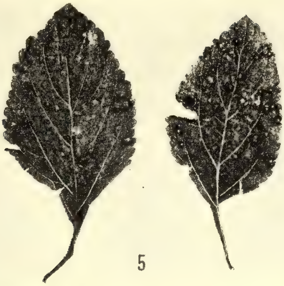
OLIVE AND WHETZEL: ENDOPHYLLUM-LIKE RUSTS.



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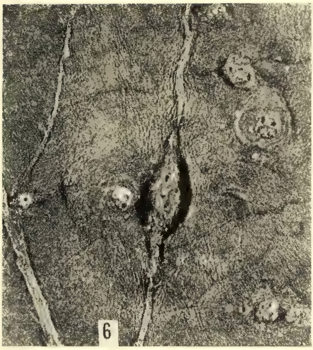

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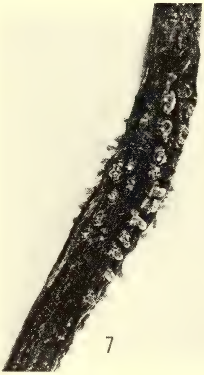

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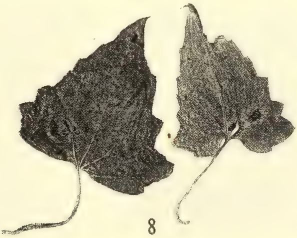

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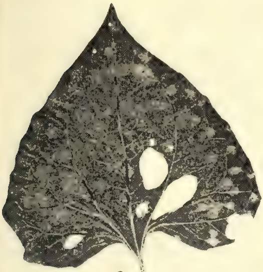
OLIVE AND WHETZEL: ENDOPHYLLUM-LIKE RUSTS.



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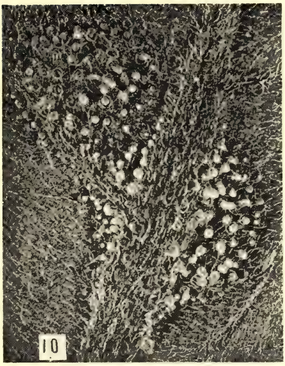

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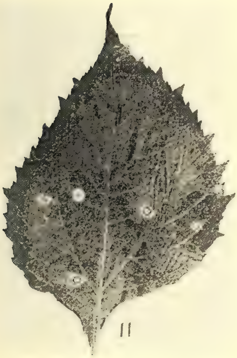

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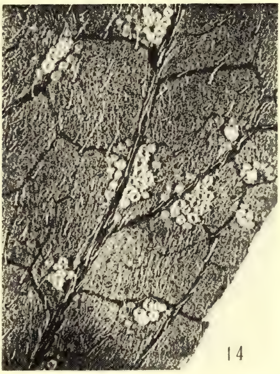

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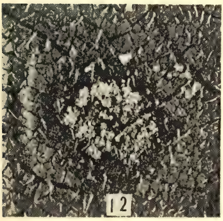

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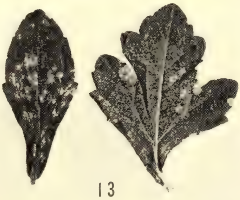

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OLIVE AND WHETZEL: ENDOPHYLLUM-LIKE RUSTS.

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CONTENTS

Fifteen- and sixteen-chromosome Oenothera mutants......ANNE M. LUTZ 53

The influence of temperature on the growth of Endothia parasitica.
NEIL E. STEVENS 112

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AMERICAN
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FIFTEEN- AND SIXTEEN-CHROMOSOME OENOTHERA MUTANTS[1]

Anne M. Lutz

A. Introduction

The present communication is the second of a series of three, the first having been published in a preceding issue of this Journal[2] under the head of "Mutants of Oenothera with diminutive chromosomes." The three papers embody a portion of the results derived from a prolonged study of the Lamarckiana group of Oenothera conducted at the Station for Experimental Evolution[3] (1907–1911), at the University of Louvain in Professor V. Grégoire's laboratory (1911–1912), and later in consultation with Professor Grégoire by letter (1912–1914).

As stated in the first report of the series, the primary object of these communications is to discuss, in the light of the Cold Spring Harbor and Louvain studies, certain theories and conclusions which Gates has given out from time to time and which Gates and Miss Thomas ('14) have based upon the results of their investigations.

The first paper described an interesting condition found in two mutant types produced by 15-chromosome *O. lata* × 14-chromosome *O. Lamarckiana;* one, a new form, *O. aberrans*, grown at Cold Spring Harbor in 1908 and 1909, and the other, *O. rubrinervis*, grown at Amsterdam in 1912. The somatic cells of these three plants were shown

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[1] Briefly reported in a paper read before the Botanical Society of America, December 29, 1915, and in a preliminary note published in Science (Lutz, '16*a*) entitled "The production of 14+-chromosome mutants by 14-chromosome *Oenothera Lamarckiana*."

[2] Amer. Journ. Bot. **3**: 502–526. 1916.

[3] Maintained by the Carnegie Institution of Washington until March, 1911.

[The **Journal** for January (**4**: 1–52) was issued Feb. 3, 1917.]

to have fourteen chromosomes of the usual size and one small one. The germ-cells were not examined. The significance of this 14^{+1}-chromosome condition in offspring of 15-chromosome mothers was discussed in relation to the discoveries of Geerts, who showed in 1911 that seven of the twenty-one chromosomes of certain hybrids may fragment and degenerate during reduction, and of Gates and Miss Thomas ('14) who demonstrated that one of the fifteen chromosomes of *O. lata* and certain *lata*-like forms may sometimes behave in a similar namner.

In the paper just referred to, Gates and Miss Thomas announced the precise somatic chromosome number of 21 plants falling under the heads of *O. lata*, *O. semilata* and various *lata*-like forms. The authors found that "all without exception contained 15 chromosomes" and have discussed many new and interesting features "in connection with the behaviour of the extra chromosome and the phenomena of degeneration." Their researches appear to have led them to conclude that the presence of the extra chromosome in 15-chromosome offspring of 14-chromosome forms is invariably associated with *lata* or *lata*-like characters in the soma of the mutant. Later Gates ('15*a*, pp. 147–148) described a 15-chromosome mutant which he showed had a few characters in common with *O. lata* and many others which were quite unlike those of the latter form. It appears, however, that he regarded this mutant as a *lata*-like form, since nowhere in this work has he intimated that the discovery of 15 chromosomes in *O. incurvata* has modified his previously expressed views concerning the relation of *lata* characters to the extra chromosome. In March of the same year de Vries ('15*a*, p. 187) described two types of offspring, besides a mutant which Stomps had obtained from *O. biennis semigigas* pollinated without castration by pure *biennis*. One of the two types, represented by 8 individuals, had 15 chromosomes and he calls attention to the fact that while these plants had the same number of chromosomes as *O. lata, they had none of the characters of the latter form.* In December following Gates ('15*b*) recognized the fact that his mutant *O. incurvata* is quite different from *O. lata*, as is also the 15-chromosome form which de Vries reported. He adds: "Hence we may say that whenever a germ cell having 8 chromosomes fertilizes a normal germ cell a new form is produced, though what its characters will be depends upon various circumstances which need not be considered here. One of the most important of these factors is probably the peculiar combination of chromosomes received." He does not state, however, that these discoveries render untenable certain earlier conclusions of Gates and of Gates and Miss Thomas concerning the relation of the extra chromosome to *lata* and *lata*-like characters, but takes what appears to be a last brave stand to save the day in the statement which follows the words quoted above: "It is perhaps not inappropriate to speak of all these mutants as belonging to the *lata* series, or the series with an extra chromosome." It must be conceded, however, that a plant which de Vries clearly states has none of the characters of *O. lata*, can not logically be held to belong to the *lata* series of mutants. Furthermore, it has been shown in the note which preceded this publication (Lutz, '16*a*) that a number of distinct mutant forms, quite unlike *O. lata*, have been found by the writer to have 15 chromosomes. The chromosome number of each of the 9 un*lata*-like types reported was ascertained, and their dissimilarity to *O. lata* fully recognized, previous to the year 1913.

The primary object of this paper, therefore, is to discuss Gates's and Gates and Miss Thomas's theories and conclusions regarding the extra chromosome at length in the light of the fact that many un*lata*-like 15-chromosome mutants are now known and that many more doubtless exist, in order that it may be shown that many of their conclusions are untenable.

B. 15-Chromosome Mutants

1. *Has* O. lata *14 or 15 Chromosomes? Is the Number of Somatic Chromosomes Inconstant in this Form?*

For a period of four or five years following the announcement of the somatic chromosome number of *O. Lamarckiana* by Geerts in 1907, all mutant offspring of *O. Lamarckiana*, with the exception of *O. gigas*, were supposed to have the same number of chromosomes as the parental form; namely, fourteen. Fifteen chromosomes had been reported for *O. albida*[4] in one of the earlier notes published by the writer (Lutz, '08), but the discovery was not emphasized and doubtless was overlooked, with the result that *O. gigas* continued to be regarded as the sole mutant derivative of *O. Lamarckiana* with a chromosome number differing from that of the parental form.

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[4] Two plants. Notwithstanding the fact that they were offspring of *O. lata* × *O. Lamarckiana*, they were mutants, since *O. albida* was not employed as either parent.

In the year following that in which the note was published concerning *O. albida*, it was announced that 15 chromosomes had been counted in two *lata* offspring of *O. lata* × *O. gigas* (Lutz, '09). These plants, of course, were not mutants, but it did not seem unreasonable to suppose that mutant *lata* would be found to have the same number of chromosomes as the hybrids. Gates, however, had repeatedly announced 14 as the sporophyte number for *O. lata* and the evidence produced seemed quite sufficient to support his claims. In a preliminary note published in 1907[5] he said (p. 260): "The sporophyte number of chromosomes in *O. lata* . . . is 14." Speaking of *O. lata* in the detailed report which followed,[6] he said (p. 92): "It has been determined *from a number of counts* in the prophase that the sporophyte number of chromosomes . . ., is 14." (Italics not employed in the original.) Again, later in 1907[7] (p. 9), "In *O. lata* the count of chromosomes was made in the pollen mother cells and found to be fourteen. It has since been made in various somatic tissues of the flower, and is found to be constantly fourteen so far as observed. *There has been no indication whatever that the number is ever higher.*" (Italics not employed in the original.) Again, on page 11, "Several plants of *O. lata* and the pure *O. Lamarckiana* have been examined, all having fourteen chromosomes."

As earlier stated, the primary object of the Cold Spring Harbor studies of Oenothera, begun in 1907, was to ascertain whether or not each particular combination of somatic characters, such as that represented by the type we know as *O. lata*, for example, is associated with a definite, fixed number of somatic chromosomes; in other words, whether or not somatic chromosome number in Oenothera is constant. Years of careful study, by the writer, of the vegetative characters of plants from seedling to fruiting stage (never overlooking the importance of taking note of the slightest deviation from the combination represented by the type) together with the precise determination of somatic chromosome number in over 200 individuals, established the fact that each combination of somatic characters is constantly associated with a certain number of chromosomes; in other words, that each type of plant has a definite, fixed number of chromosomes. It was therefore announced in "Triploid mutants" (Lutz, '12), and many times emphasized throughout the report (pp. 390, 416, 418, 420) that *all individuals having identical somatic characters from seedling to fruiting stage invariably have identical somatic chromosome numbers, regardless of the parentage or the origin of the plants in question.* Owing to the contradictory nature of the evidence produced by Gates and Lutz relating to somatic chromosome number in *O. lata*, it was feared that this type might be regarded as an exception to the rule.[8] It was therefore stated (p. 416) that the Cold Spring Harbor studies had established the precise somatic chromosome number of 28 latas; that each had been found to have 15 chromosomes, "whether mutant, hybrid, offspring of mutant *lata* self-pollinated, or offspring of hybrid *lata* self-pollinated; whether grown at Amsterdam, Cold Spring Harbor, or the New York Botanical Garden; and whether derived from de Vries's cultures, from plants descended from plants or seeds from de Vries, or from plants of English ancestry, in no wise related to de Vries's cultures."

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[5] Gates, '07*a*.

[6] Gates, '07*b*.

[7] Gates, '07*c*.

Later in the same year in which the above announcement was made, Gates ('12) stated that he had counted the chromosomes in one *lata* plant and had found the number in this individual to be 15, and added (p. 995): "From all the counts thus far made of the chromosomes in *O. lata* it appears that the number 15 occurs at least in most individuals, though the counts are perhaps not yet numerous enough to show that 15 is the number for all individuals, . . ." From a note added at the conclusion of this report, it seems that the statement concerning *O. lata* in "Triploid mutants" had not appeared in print or had not attracted his notice at the time this communication was sent to press. In the note he says that the preparations of *O. lata* described in his first paper on the subject were re-examined, but that they had deteriorated somewhat and he was "unable to determine with certainty whether this *lata* plant contained 14 or 15 chromosomes."

Since Gates's early statements were clearly based upon a number of counts, it seems improbable that the extra chromosome, if present, would have repeatedly escaped his notice. Owing to an unfortunate error in identification during the first year of the work, Lutz ('08) had announced 14 chromosomes for a plant supposed to be *O. lata*, but later shown to be a distinct type.[9] It is possible, therefore, that Gates mistook some *lata*-like form having 14 chromosomes, for *O. lata*. The number of individuals in which he counted 14 chromosomes is not known; it is clear from the note referred to at the end of the preceding paragraph, that only one plant was mentioned in the first two 1907 papers, but his statements in the third that "Several plants of *O. lata* and the pure *O. Lamarckiana* have been examined, all having fourteen chromosomes," certainly indicates that 14 had been counted in more than one individual identified as *O. lata*. At any rate, Gates appears to be convinced of error in count or identification in his early studies of *O. lata*, since he states ('13, pp. 301–302) that Gates and Miss Thomas's studies of *O. lata*, etc. "corroborated the independent results of Miss Lutz and Gates regarding the constancy of the fifteen chromosomes in *O.* mut. *lata*, . . ." Furthermore, Gates and Miss Thomas ('14) not only emphasize the constancy of the 15-chromosome condition in *O. lata* without reference to the earlier count of 14, but appear to be convinced that plants having 15 chromosomes invariably have *lata*, *semilata*, or *lata*-like characters. In fact, in Gates's recent work ('15*a*, pp. 167 and 296) he says, in referring to the *lata* plant discussed in his first two 1907 reports, that his discoveries indicated

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[8] One may be led to inquire also whether 14^{+1}-chromosome forms may not be regarded as exceptions to the rule. In considering this question it should be borne in mind that we do not yet know whether the small member of the chromosome group is constant or variable. Should future studies show the latter to be true, we should then be called upon to decide whether these 14^{+1}-chromosome forms should be regarded as actual exceptions to the rule, since the small body is, in all probability, not a chromosome, but merely a detached fragment of a whole chromosome, or a remnant of a degenerating chromosome.

---

[9] Certain forms which were studied during the first years of the writer's work are now known to have been erroneously classified (see '12, p. 390, note 11, and 16 *b*, p. 514, note 7). The reappearance of the 14-chromosome form supposed to be *O. lata* has shown that this mutant was not *O. lata*, though resembling it strongly in early rosette characters (to be demonstrated in a later publication). The 16th chromosome of one figure of a second *lata* has since been demonstrated to me by Professor Grégoire to be merely a deceptive anastomosis between two parallel chromosomes although Gates ('12) has since reported two 16-chromosome cells in a 15-chromosome *lata* and one 16-chromosome cell has also been found in a C. S. H. *lata*. The 15-chromosome form called *O. nanella* was a dwarf, not *O. nanella*. Likewise, it has since been demonstrated that the 14- and 15-chromosome forms designated as *oblonga* did not duplicate each other and that the first type is certainly not *oblonga*, though *oblonga*-like, and the second (type 5509) may be *oblonga* but is believed to be a modified form of de Vries's mutant. The latter type has continued to appear in *Lamarckiana* and other cultures since 1908. These errors of identification were the result of premature publication of inexperienced work and are most regretable, as they serve only to mislead others. By withholding later publications until identifications and results of investigations could be verified, it is hoped that similar errors have been avoided.

"about" 14 chromosomes, but that the number for *O. lata* has since been shown by Lutz, Gates, and Gates and Miss Thomas, to be 15.

These facts are outlined at length in order that the reader may understand that mistaken identification of one plant by Lutz and possible mistaken identification or error in count by Gates are responsible for the early statement that *O. lata* had 14 chromosomes. It may be assumed with safety now that the number of somatic chromosomes present in the type of *O. lata* produced by *O. Lamarckiana* is invariably 15.

2. O. lata *and the "Extra" Chromosome*

In "Recent papers on Oenothera mutations" Gates ('13, pp. 301–302), as stated above, mentions the then unpublished results of investigations conducted by Gates and Miss Thomas ('14) which had disclosed 15 chromosomes in 21 plants classified by them as *O.* mut. *lata*, *O.* mut. *semilata*, *O. lata* to *semilata*, *O.* mut. *lata rubricalyx*, *O. biennis* mut. *lata* and as *lata*-like forms. Referring to *O. lata rubricalyx* which appeared among the F_2 offspring of two 14-chromosome forms crossed (*O. rubricalyx* × *O. grandiflora*), he says: "The possession of fifteen chromosomes by this plant also shows that whenever a meiotic irregularity leads to the formation of an individual having an extra chromosome, such a plant will have the leaves and habit of *lata* or *semilata*." Although he adds in a footnote that "It is possible that one or two other mutants also have an extra chromosome," he does not state or intimate that such forms are not *lata*-like; furthermore, Gates and Miss Thomas say in the later report (pp. 551–552), "Certain other mutants indicate by their hereditary behaviour that they may also have aberrant chromosome numbers, but this has not yet been proved, except in *gigas*."

Gates was the first to show that one of the heterotypic chromosomes of a form may pass "into the same daughter-nucleus as its mate, instead of into the opposite nucleus." He first demonstrated this significant irregularity in 14-chromosome *Lamarckiana* in 1907, but has since observed the same peculiarity in many other forms. With reference to this occasional 6–8 distribution of heterotypic chromosomes in 14-chromosome forms, Gates and Miss Thomas say (p. 550): "Whenever this irregular meoitic division occurs in a pollen mother-cell, such a cell will, at least in many cases, give rise to two *lata*-producing pollen grains in addition to two having only 6 chromosomes. The latter apparently always degenerate. Similarly, when such an irregularity occurs in the megaspore meiosis, if the 8-chromosome megaspore functions it will, after fertilisation by a 7-chromosome pollen grain, give rise to a *lata*-like mutant. . . . Moreover, in *lata* or *semilata* when crossed with their 14-chromosome parents or when self-pollinated, the percentage in which the mutant reappears will depend upon the relative number of their 8-chromosome and 7-chromosome germ-cells which function."

The authors then state that the frequency of this unequal division appears, from the observations of Gates to be "of the order of 1 per cent." This, they say, would give about two 8-chromosome pollen grains in 400, or 0.5 percent, and that "If the frequency of this irregularity in the megaspore mother-cells is the same, about 1 per cent. of *lata* mutations should be anticipated."

Gates's claim that "whenever a meiotic irregularity leads to the formation of an individual having an extra chromosome, such a plant will have the leaves and habit of *O. lata* or *O. semilata*" leaves no loophole for escape from the conclusion that all 15-chromosome offspring of 14-chromosome forms—or at least all which are derived from the fertilization of an 8-chromosome egg by a 7-chromosome sperm—"have the leaves and habit of *O. lata* or *O. semilata*," while Gates and Miss Thomas's estimates of the percentages of offspring of *O. lata*, selfed, and of *O. lata* × *O. Lamarckiana* which may be expected to reproduce the characters of *O. lata*, lead the reader to conclude that progeny resulting from 8 + 7 unions invariably have the characters of *O. lata*, *O. semilata*, or some *lata*-like form.

All of the 15-chromosome mutants which Gates and Miss Thomas mentioned in this report and which Gates has discussed in earlier publications, were classified as *O. lata*,[10] *O. semilata*, *lata* to *semilata* or *lata*-like forms, and it appears that these were the only 15-chromosome mutants whose somatic chromosome numbers had been ascertained by them at that time;[11] if such be the case, this chance occurrence is probably responsible for their conclusions. That many of the 15-chromosome mutant offspring produced by 14-chromosome forms have *lata* or *lata*-like characters cannot be questioned, but it is equally certain that a far greater number do not. It seems to the writer that the authors' announcements were a bit premature in view of the fact that the somatic chromosome numbers of so many mutant offspring of 14-chromosome *O. Lamarckiana* had not been reported. For example, the somatic chromosome numbers of *O. scintillans*, *O. spathulata*, *O. elliptica*, *O. sublinearis*, *O. leptocarpa*, *O. subovata*, etc., all *Lamarckiana* mutants, had not been announced at that time. The same is true of *O. nanella lata*,[12] *O. nanella oblonga* and *O. nanella elliptica*—compound types appearing in 14-chromosome *O. nanella* cultures and elsewhere. Furthermore, as previously stated, it has been shown (1908) that *O. albida*, one of the very common *Lamarckiana* mutants, is a 15-chromosome form, yet this plant cannot be listed either as *O. lata* or as a *lata*-like form.

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[10] Including *O. lata rubricalyx* and *O. biennis lata*.

[11] As we have seen, Gates ('15*a*) has since reported a 15-chromosome mutant, *O. incurvata*, which still later ('15*b*) he says is quite different from *O. lata*.

I have studied the somatic chromosomes of 305 plants of the *Lamarckiana* group and have determined the precise number of 234 individuals. Exclusive of the two 14^{+1}-chromosome mutant types mentioned in the preceding report, of the offspring of *O. lata* × *O. gigas*, *O. Lamarckiana* × *O. gigas*, *O. nanella* × *O. gigas* and of *O. gigas*, selfed, *16 distinct mutant types were found among these 234 individuals (17 among the 305) having more than 14 chromosomes; 11 of the 16 were 15-chromosome forms, 3 were 16- and 2 were triploid. The 17th type having 14+ chromosomes was also a triploid form.* In addition to the above, one type having a number of characters in common with one of the eleven 15-chromosome types mentioned, was also found to have 15 chromosomes. Also, 15 chromosomes were repeatedly counted in root-tips from a mutant grown at Amsterdam in 1912 and identified by Professor de Vries as *O. oblonga*, but a certain irregularity found in several tips of the plant (to be described later) indicated a possible abnormal condition and made it seem inadvisable to accept the count in this individual as typical of the species until verified by counts in other oblongas.[14] Besides the 11 or 12 types which were ascertained,

[12] Hunger ('13) found plants which he identified as *O. lata nanella* and *O. oblonga nanella* in cultures of *O. Lamarckiana*.

[13] I have since verified this count in 13 additional *albida* mutants.

[14] Professor de Vries has kindly aided me in every way to determine the precise number of chromosomes in the Amsterdam type. In the early summer of 1915 he sent me a number of young *oblonga* rosettes from his gardens, but, unfortunately, all perished before reaching their destination. He also sent me a generous supply of seeds from one of his best plants, but very few of these germinated and only one seedling survived. Root-tip fixations were prepared from this plant, but no satisfactory counts have been obtained from them thus far.

beyond doubt, to have 15 chromosomes, 2 quite distinct types had 15(?) chromosomes (number not determined precisely).

We may now consider, briefly, the evidence furnished by these 15- and 15(?)-chromosome forms.

(*a*) *Distinct Types Having 15 Chromosomes.*—Four of the 11 distinct types are very common *Lamarckiana* mutants, though found in other cultures, as well: (1) *O. lata*, (2) *O. albida*, (3) *O. bipartita* (C.S.H.) and (4) type 5509 (C.S.H.), supposed to be a modified form of de Vries's *oblonga*. Among the less common forms are (5) *O. nanella lata*,[15] obtained from de Vries's culture of *O. lata* × *O. Lamarckiana* (1912), but found also in cultures of *O. Lamarckiana*, *O. nanella*, etc. (6) *O. subovata*, obtained from *O. lata* × *O. Lamarckiana*, but also produced by *O. Lamarckiana*. (7) A dwarf mutant, type 2256, found in a culture of *O. nanella*, (8) type 4499, produced by *O. lata*, selfed, and *O. lata* × *O. Lamarckiana;* and three mutants which have been observed in *lata* cultures only, thus far: (9) *O. exilis*, (10) *O. exundans* and (11) type 5365.

O. bipartita is a remarkably beautiful and interesting form. The peculiarities of the young plant not yet come to flower are shown in Figure 1. The leaves, particularly those of the young plants, are thin and papery feeling; those of the adult form being more crinkled and more finely crinkled, being somewhat broader in proportion to their length, than *Lamarckiana* leaves. Although *bipartita* attains the height of the tallest *Lamarckiana*, it is more dainty in appearance than the parental form (Fig. 2). Like the latter, it produces a circlet of basal branches which are somewhat shorter and less decumbent than the rosette branches of *Lamarckiana* (Fig. 3). Not only are the branches more slender, but the buds, which are regular and tapering, are shorter and the flowers smaller than in the case of *Lamarckiana*.

O. bipartita is distinguished by the large number of flowers produced having more than 4 regular, tapering, stigmatic rays. Flowers with 4+-rayed stigmas are common to most forms, yet the number of flowers produced daily by *bipartita* having 4+-rayed stigmas forms a higher percentage of the total than has been found to be true of any other one of the freely blooming plants. The percentage of flowers having 4+-rayed stigmas varies greatly among the individuals of a given type. Daily records were made during the greater part of a

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[15] Professor de Vries states that he uses the term "*O. lata nanella*" and "*O. nanella lata*" interchangeably.

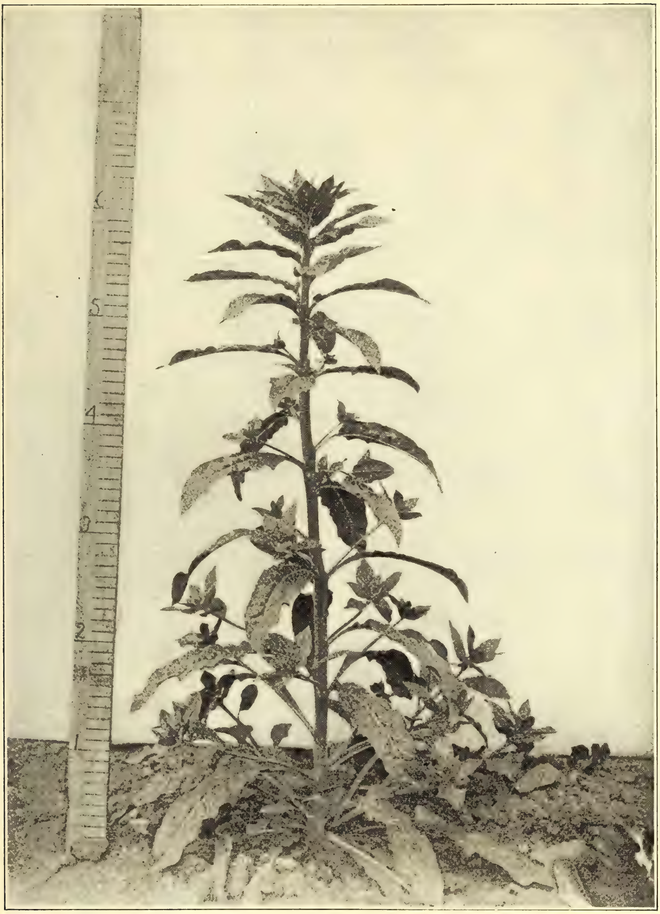

FIG. 1. *O. bipartita*, plant No. 5561. C.S.H., 1910. Mutant offspring of *O. Lamarckiana* × *O. Lamarckiana*. Not yet come to flower.

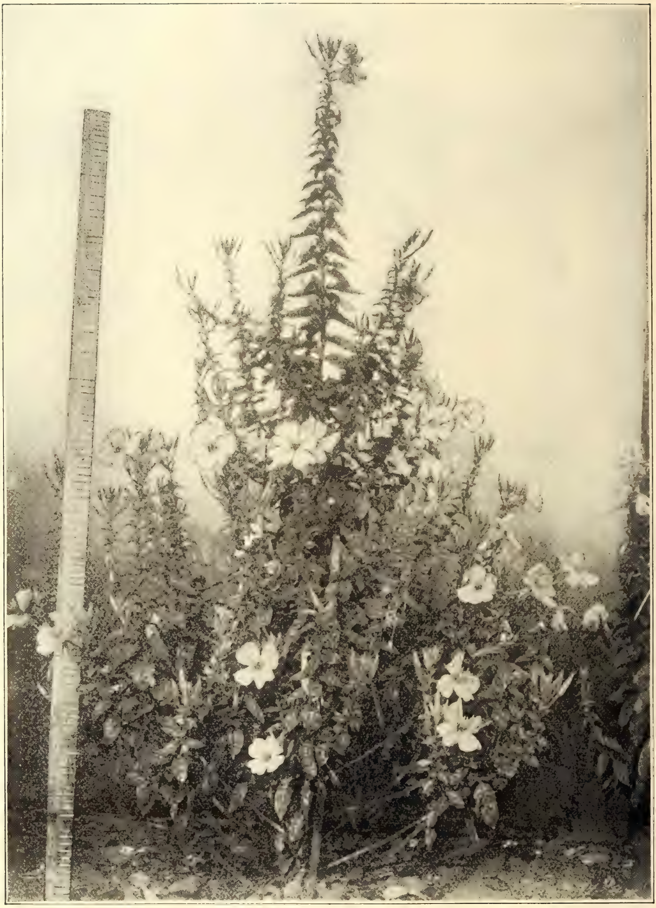

FIG. 2. *O. bipartita,* plant No. 5561, at height of flowering period. One of the two uppermost flowers shows cleft petal.

flowering season of the flowers produced by two biennial Lamarckianas, and it was found that a larger percentage of the early, than of the late, flowers had 4+-rayed stigmas.

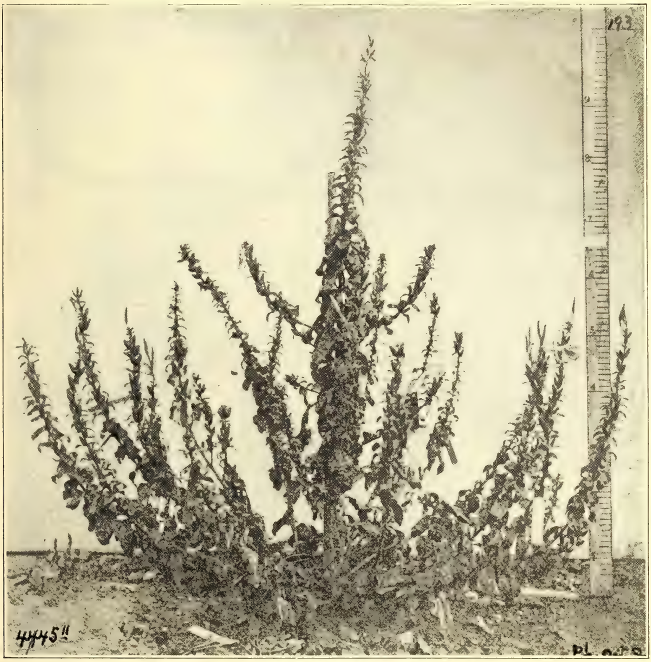

FIG. 3. *O. Lamarckiana*, plant No. 5958, C.S.H., 1909. Offspring of *O. Lamarckiana*, selfed. Photographed late in the season to show manner of branching.

This seemed to be due to a tendency on the part of the first flowers of the stem and vigorous rosette and stem branches to have 4+-, rather than 4-rayed stigmas, though flowers with 4-rayed stigmas were common among the first, and flowers with 4+- were frequently found near the terminal portions of these parts. After the plants had bloomed a short time, it was found that the number of flowers having 4-rayed stigmas exceeded the number of those having 4+-, and towards the close of the flowering season it was seen that the number of the former greatly exceeded that of the latter.

On a certain day, at the height of the flowering season of 1910, 62 flowers unfolded on one *bipartita* mutant, and 52 of these had 4+-rayed stigmas. On August 31 of the same year, 80 percent of the 214 flowers produced by 9 *bipartita* mutants had 4+-rayed stigmas, while less than 1 percent of the 312 produced by 9 Lamarckianas selected at random on the same day, were distinguished in this manner. All had been in flower about the same length of time.

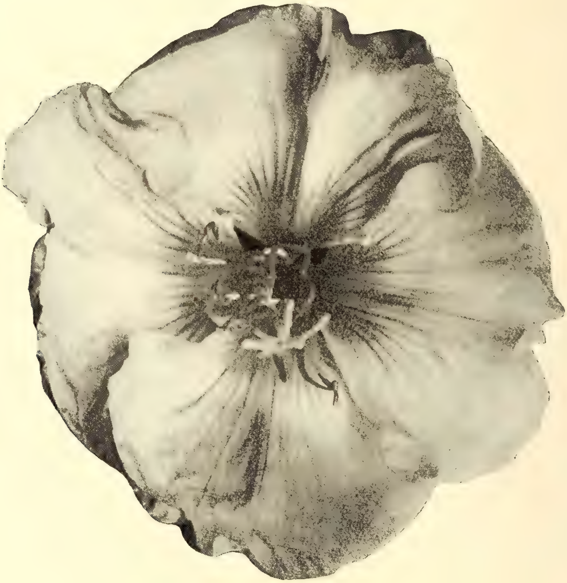

FIG. 4. F_2 *O. lata* × *O. gigas*, plant No. 4930, C.S.H., 1909. Flower showing normal arrangement of petals.

When a bud is held with the apex of the cone upward and the sepals are then stripped backward, it will be found that the petals are rolled, in the majority of cases, from left to right (viewed from the sepal side) with the right lateral margin free and the left overlapped by the right lateral of the preceding petal (Fig. 4). Occasionally, when a bud opens, it is found that the relative positions of the right and left margins of two neighboring petals are reversed, the left of one overlapping the right of the other. A few buds have been found in which a complete reversal of relative positions had occurred at all four points; commonly this takes place at but one. Complete reversal produces no disturbance, but partial reversal frequently, though by no means invariably, causes interference in the growing bud. For example, when the left lateral margin of a petal, which we may designate as 2, becomes dis-

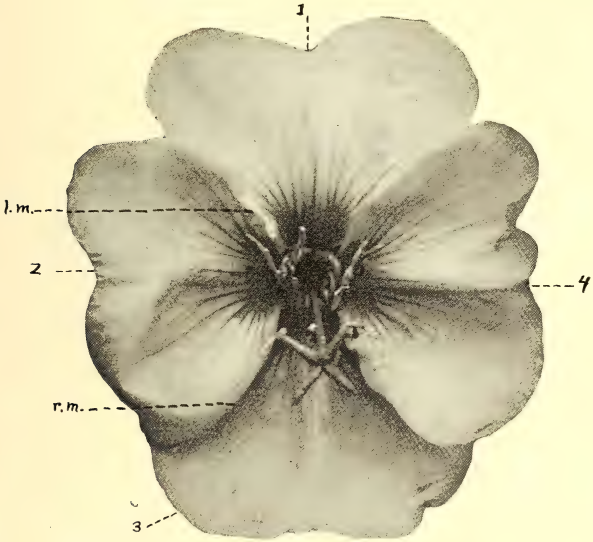

FIG. 5. F_1 *O. Lamarckiana* × *O. gigas*, plant No. 3750, C.S.H., 1908. Flower showing reversed petal arrangement at two points, the left lateral margin of petal 1 (*l. m.*) overlapping the right lateral of 4 (*r. m.*) and the left of 3 overlapping the right of 2, leaving petals 2 and 4 in, and 1 and 3, out.

placed and overlaps the right lateral margin of petal 1, both lateral margins of petal 1 are left in (Fig. 6), and both of petal 2, out. Therefore the left of 2 and right of 4 are both out, and in the growing bud sometimes forms a sort of an X contact along the left distal margin of 2 and right distal of 4.[16] The right distal of 4 may grow on

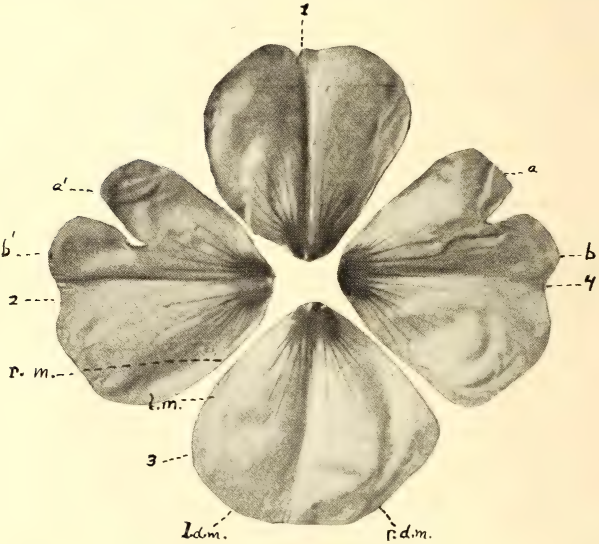

FIG. 6. F_2 *O. gigas* × *O. Lamarckiana* (de Vries), plant No. 133(1), Lafayette, Indiana, 1913. Detached petals of flowers showing petal cleavage resulting from reversed petal arrangement at one point, the left lateral margin of petal 2 overlapping the right lateral of 1, leaving both margins of 1 in, and both of 2, out, in open flower. Most common form of irregular arrangement and petal cleavage. *a, b* = right distal lobes; *a′, b′* = left distal lobes; *r.m.* = right lateral margin; *l.m.* = left lateral margin; *l.d.m.* and *r.d.m.* = left and right distal margins.

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[16] Since Figs. 4, 5 and 6 show flowers photographed from the stigma, instead of from the sepal, surface, the margins which are referred to as right and left in the descriptions of the bud appear in reversed positions when viewed from the inside of the open flowers. The margins are labeled, however, as seen from the sepal surface of the bud.

both sides of the left distal of 2, the latter, as it grows, being crumpled into the slit thus produced by 4. Stripping back the sepals of a bud about to flower, one will find the two petals locked in this manner, the *a* lobe of 4 overlapping the *a′* of 2, and the *b′* lobe of 2 overlapping the *b* of 4.[17] When the flower unfolds, petal 4 may be found with a diagonal slit extending from about the middle of the right half of the distal margin towards the median line of the petal. Petal 2 may or may not have a slit extending from about the middle of the left distal margin towards the median line. It is usually present, and shorter than the slit in petal 4. In the case of the flower shown in Fig. 6, petal 2 has the longer incision, indicating that lobe *a′* of petal 2 overlapped lobe *a* of petal 4, and that lobe *b* of 4 overlapped *b′* of 2. Flowers are found with one, two, three, or all four, of the petals cleft. Sometimes a petal has two slits, one on each side of the median line. In an irregularity such as that first described above, the right margin of 1, as well as the left of 3, is in, consequently these two sometimes interfere, causing an incision either in the left distal margin of 3, or the right distal of 1, or both. Sometimes petal 1, both margins of which are in, wraps around the filaments and anthers. The right and left distal margins may then come in contact in such a way as to cause the two incisions sometimes found in a petal. Not infrequently an irregularity in petal arrangement causes no cleavage. Although cleft petals are sometimes produced by overhanging anthers, in the majority of cases they result from interferences caused by a partial reversal of the direction in which the petals are normally rolled within the bud.

O. bipartita is distinguished by the large number of flowers produced with cleft petals. Flowers with cleft petals are found occasionally on individuals of almost any type, including *Lamarckiana*, but they are more common to *bipartita* than any other mutant type observed.[18]

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[17] The petal whose base is attached at the point where the filaments separate is designated as petal 1.

[18] The first flowers of vigorous rosette and stem branches (probably also of the stem) appear to be more subject to this irregularity than those produced by the same parts near the extremity. It is possible also that the first flowers of the short, weak secondary branches produced late in the flowering season are less subject to this irregularity than the first flowers of more vigorous parts, but the facts have not been ascertained regarding this point. The buds of two biennial Lamarckianas were examined daily (with occasional exceptions) throughout the greater part of the flowering season. Plant *A* came to flower June 23 and *B* about the same time. The former was examined for cleft-petaled flowers for the first time on June 29, and the latter on June 28. The results for these days and the 11 and 12 following, were recorded as follows:

Scarcely a day but one or two cleft-petaled flowers were found on each plant, and usually many more. On a certain day, 22 of the 45 flowers produced by one of these mutants had cleft petals. The records for the 9 mutants employed for 4+-rayed stigma counts (see page 66) on the day previously mentioned, August 31, will serve as a typical illustration: 74 of the 214 flowers produced, almost 35 percent, had cleft petals.

The pollen of *O. bipartita* consists of 3-lobed grains. In the buds of the mutants observed it was found to be entirely absent, produced in small quantities, or present in moderate amounts; these conditions were found in the various buds of each plant. A large percentage of the grains produced are bad, and it is exceedingly difficult to obtain seeds from these forms, selfed.

Type 5509, presumably a modified form of de Vries's *oblonga*, seems to bear about the same relation to the Amsterdam mutant as type 3514 (see Lutz, '16*b*) bears to de Vries's *rubrinervis;* yet it is possible that the two are identical forms.

(*b*) *A Related Type, Having 15 Chromosomes.*—Type 2806, a form having many points in common with type 5509. Also found in cultures of *O. Lamarckiana.*

(*c*) *Distinct Types Having 15*(?) *Chromosomes.*—These are (1) a plant from de Vries's 1912 culture of *O. lata* × *O. Lamarckiana,* said

	A			*B*		
	Total Number of Flowers	Number with Irregular Petal Arrangement	Number with Irregular Petal Arrangement and Cleft Petals	Total Number of Flowers	Number with Irregular Petal Arrangement	Number with Irregular Petal Arrangement and Cleft Petals
June 28.....	—	—	—	30	13	8
“ 29.....	54	3	3	52	4	2
“ 30.....	67	5	5	70	4	4
July 1.....	63	0	0	82	3	3
“ 2.....	—	—	—	100	10	0
“ 3.....	120	4	1	74	0	0
“ 4.....	113	—	—	90	—	—
“ 5.....	132	1	1	80	0	0
“ 6.....	99	1	1	59	0	0
“ 7.....	59	0	0	45	0	0
“ 8.....	—	—	—		—	—
“ 9.....	—	—	—		0	0
“ 10.....	22	0	0		0	0

From July 10 until the close of the flowering season cleft-petaled flowers continued to appear occasionally, but much less frequently than during the early flowering period of the plants.

to have combined the characters of *O. lata* with the smooth, shining leaves of *O. Leata*. (2) *O. elliptica*, a *Lamarckiana* mutant.

Of the 11 distinct types known to have 15 chromosomes, (1) *O. lata*, (2) *O. albida*, (5) *O. nanella lata* and (6) *O. subovata* are well-known forms, originally described by de Vries; (4) type 5509 was mentioned in an early note (Lutz, '08); (7) type 2256, as previously pointed out, was erroneously identified in 1908 as *O. nanella*. All of the remaining five forms are new. The somatic chromosome numbers of *O. lata*, *O. albida*, types 5509 and 2256 were reported in early notes by Lutz; those of *O. bipartita*, *O. nanella lata*, *O. exilis*, *O. subovata*, *O. exundans*, types 4499 and 5365 were communicated in the preliminary note to this paper (Lutz, '16*a*).

A mutant of the *Lamarckiana* group is distinguished from the parental form and other mutants, not so much by some particular character—for few characters are peculiar to any one type alone—as by the combination of characters which is peculiar to itself. Thus, *O. lata* has broad, heavily crinkled leaves, irregularly shaped buds (particularly true of the early buds), light yellow flowers with crumpled petals, barren anthers, etc. No one of these characters is peculiar to *O. lata* alone. The cleft petals and large percentage of flowers having 4+-rayed stigmas are striking characters of *O. bipartita*, yet neither is peculiar to this form alone; it is the combination of characters previously enumerated which distinguishes it from all other forms. A very striking illustration of this point may be found in the previously mentioned 15-chromosome mutant reported by Gates ('15*a*, pp. 147–148), namely, *O. incurvata*. His illustrations and descriptions of this form clearly show that it is not entitled to be regarded as a *lata*-like form, for, although he states that it agrees with *O. lata* "in the obtuse tips and deep crinkling of the leaves," he also says that it differs from *O. lata* "(1) in the much narrower leaves with long petioles, (2) in having one edge of the leaf characteristically folded over, (3) in being as tall as *Lamarckiana* with long internodes, (4) in having more squarish buds which produce pollen." If, in connection with these statements, one compares his photographs of *O. incurvata* (Figs. 56 and 57) with that of *O. lata* (Fig. 37), all in the rosette stage, one will see that *O. lata* and *O. incurvata* are about as unlike as any two mutants which may be mentioned. It is quite clear that the "obtuse tips and deep crinkling of the leaves" do not entitle this form to be regarded as *lata*-like, since it is wholly unlike

O. lata in the majority of its characters. The tendency of the margin of the leaves of *incurvata* to roll towards the upper surface of the midrib is one of the most striking characteristics of the full-grown rosette leaves of *O. albida* (compare Fig. 7 with Gates's Fig. 56).

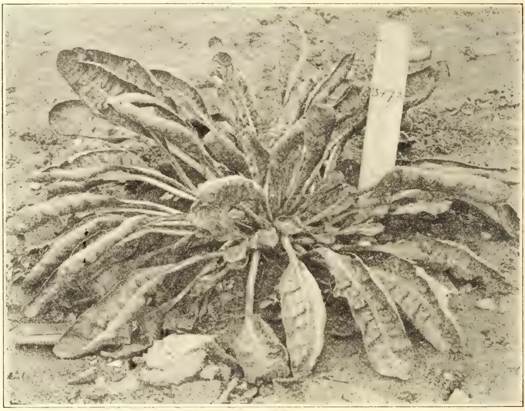

Fig. 7. *O. albida*, plant No. 3472, C.S.H., 1908. Offspring of *O. Lamarckiana* × *O. Lamarckiana.* Mutant in late rosette stage showing margins of leaf blade rolling towards the midrib; a typical *albida* character.

In view of the above facts, it is not surprising to find that a few of the twelve 15-chromosome types had one or two characters suggestive of *O. lata*, just as others had one or two suggestive of *O. Lamarckiana*, of *O. rubrinervis* or some other form; yet, since the majority of the characters were wholly unlike those of *O. lata* in the first case and wholly unlike those of *O. Lamarckiana* and *O. rubrinervis* in the latter instances, the first could not be called *lata*-like nor the latter *Lamarckiana*- or *rubrinervis*-like. In fact, only 2 of the 12 types were *lata*-like; namely, *O. lata* and *O. nanella lata.* On the other hand, *2 plants (2 types) were found in Cold Spring Harbor cultures of* O. Lamarckiana, *which were conspicuously* lata-*like in appearance, though differing from* O. lata *sufficiently to be regarded as distinct forms, and each had 16, and not 15, chromosomes.*

De Vries ('09, Vol. I., p. 224) gives a table showing the origin of new species from the type, *O. Lamarckiana.* From a first generation of 9 Lamarckianas he records 7 generations of descendants through *O. Lamarckiana* (1886–87 to 1899, inclusive) consisting of a total of 54,334 individuals, of which 834 were mutants, as follows:

TABLE I

Species	*O. Lamarckiana* 14	*O. nanella* 14	*O. rubrinervis* 14 or 14^{+1}	*O. lata* 15	*O. albida* 15	*O. scintillans* Prob. 15	*O. oblonga* 14 or 15	*O. gigas* 28
Number of individuals...	53,500	158	32	229	56	8	350	1

Although the somatic chromosome number of *O. scintillans* is probably 15, the actual number is unknown, hence this type will be excluded from our calculations. If *oblonga* has 14 chromosomes, we see that approximately 20 percent of the total number of 15-chromosome forms were neither *O. lata* nor *lata*-like individuals. If *oblonga* has 15, then we see that almost 64 percent were neither *O. lata* nor *lata*-like forms. But de Vries states that the list is incomplete, as only the more important mutants were recorded; furthermore, since the records date from 1886, it is probable that even the common types were occasionally overlooked in the early years of the work; in fact he says (p. 229) that *albida* was passed by as a diseased form in 1888 and 1890. For these reasons the records of his 1895 *Lamarckiana* cultures are perhaps more significant (pp. 262–263). Of the total of 10,614 offspring of *O. Lamarckiana* he states that 614, or about 6 percent were mutants, "of which *O. albida* made up 2.5 %, *O. lata* 1.7 %, *O. nanella* 1.1 %, *O. oblonga* 0.7 % and the rest altogether 0.1 %." If we include *oblonga* and the unnamed mutants among the 15-chromosome forms, we see that the total number of *albida* plants alone equalled the total number of all other 15-chromosome mutants listed, including *O. lata.* We will assume that some of the unnamed types had 15 chromosomes and others 14 and that *oblonga* also had 14. If such were the case, the number of albidas not only exceeded the number of latas, but exceeded the combined number of 15-chromosome forms not listed as *O. albida.*

It is well known that a large percentage of Oenothera seeds fail to germinate in the short time commonly allowed them when sown in seed pans in January. De Vries ('15*a*) and Davis ('15*a*) have emphasized this fact recently and have suggested means of greatly increasing the percentage of germinations. ". . . we can not feel confident," Davis states ('15*b*), "that the records of any cultures of *Oenothera* so far reported are complete for their possible progeny. The percentages calculated for 'mutants' and the ratios of classes in breeding experiments can not be accepted as final in exact genetical work. We are not in a position even to guess what may be the changes of front when exact data become available." It is clear that future records of cultures, to be of value, must show that they are complete for their possible progeny.

As a rule, seeds are obtained in greater abundance from 14-chromosome forms selfed, or pollinated by other 14-chromosome forms of the same, or different species, than from 14+-chromosome forms selfed, or pollinated by other 14+-chromosome plants of the same, or different species,—particularly if the 14+-chromosome individuals have more than 14, but fewer than 28, chromosomes.

Beginning with the summer of 1908, I adopted the practice of counting all seeds sown; of planting seeds at spaced intervals in seed pans, and of recording the germinations. Only in a few instances have these precautions been neglected. The results have clearly shown that when seeds not more than one year old are sown in pans of sterilized soil in January and kept under ordinary greenhouse conditions, usually *larger percentages of germinations are secured within the first four or five months from seeds of 14-chromosome plants selfed, or pollinated by other 14-chromosome plants of the same, or different species, than from 14+-chromosome plants selfed, or pollinated by other 14+-chromosome plants of the same, or different species—particularly if the 14+-chromosome individuals have more than 14, but fewer than 28, chromosomes.* Hence it appears that *the number of seeds produced by a form and the ability of the seeds to germinate, at least within the time limits specified, are factors which are associated with the chromosome number of the plant, or numbers of the plants, producing them. The ability of a seed to germinate appears to depend, not wholly, but to a certain extent, upon the number of chromosomes which it bears, and, possibly, in accordance with Gates's suggestion* (15*a*, p. 194), *upon the compatibility, or incompatibility of the chromosomal combination which the number represents.* It also appears that *the ability of a seed to germinate is directly associated with its own chromosome number and only* *indirectly with that of its parents*, for the results derived from the Cold Spring Harbor and Louvain studies indicate that 14-chromosome offspring of 14+-chromosome forms may germinate as readily as 14-chromosome seeds of 14-chromosome forms.

In the discussions of this report and others to follow, due allowance will be made for the facts above mentioned. With reference to the relative percentages of the various types of mutants appearing in de Vries's cultures, as quoted, these may not represent the actual percentages as they would have appeared had all the viable seeds sown germinated, but they offer sufficiently satisfactory evidence to prove that many 15-chromosome offspring of 14-chromosome *Lamarckiana*, alone, have neither *lata*, *semilata*, nor *lata*-like, characters; furthermore, it is probable that the majority of these 15-chromosome mutants, whether *lata*-like or not, are products of 7 + 8 unions. However, if we believe that each 15-chromosome mutant is represented by a particular chromosomal combination, then we will agree that an occasional 6 + 9 union might produce the same combination as 7 + 8 and that the same type of mutant might result from the former as from the latter. This possibility may be illustrated very simply.

Throughout this paper and in future reports, when we wish to indicate difference in sex without designating the particular sex of either gamete, we shall employ small capitals in italics to represent the chromosomes of a gamete of one sex and the same, marked ′, to indicate the chromosomes of a gamete of the opposite sex; thus, (♂ or ♀) *A B C D E F G* + (♂ or ♀) *A′ B′ C′ D′ E′ F′ G′* = *AA′ BB′ CC′ DD′ EE′ FF′ GG′*. When it shall be necessary to indicate the sex of gametes, the chromosomes of the female will be designated by lower case letters in italics, and those of the male by the same, marked ′; thus, ♀ *a b c d e f g* + ♂ *a′ b′ c′ d′ e′ f′ g′* = *aa′ bb′ cc′ dd′ ee′ ff′ gg′*. Now, assuming that the regular female gamete of *O. Lamarckiana* contains *a b c d e f g* chromosomes and the regular male *a′ b′ c′ d′ e′ f′ g′*; that the somatic cells of this form contain *aa′ bb′ cc′ dd′ ee′ ff′ gg′* chromosomes; then *aa b c d e f g* + *a′ b′ c′ d′ e′ f′ g′* might produce *O. lata* having *aaa′ bb′ cc′ dd′ ee′ ff′ gg′* chromosomes. So also might *aa bb c d e f g* + *a′–c′ d′ e′ f′ g′* produce *O. lata* having *aaa′ bb cc′ dd′ ee′ ff′ gg′* chromosomes. While it is possible that a 6-chromosome cell is incapable of functioning in union with one having 7 chromosomes, or fewer, but is capable of functioning in union with one having 8 or 8+ chromosomes, thereby producing a 14- or 14+-chromosome condition (Lutz, '12, p. 424), it cannot be assumed with safety that these common 15-chromosome *Lamarckiana* mutants result from the fusion of 6- and 9-chromosome gametes, except, possibly, in rare instances, for 5–9 distributions of heterotypic chromosomes doubtless occur still more rarely than 6–8, and a 9-chromosome cell would be expected to unite with a 7- far more frequently than with a 6- and to produce a 16-chromosome mutant; yet 16-chromosome offspring of *O. Lamarckiana* × *O. Lamarckiana* appear to be comparatively rare.

Gates ('09*a*, pp. 4–5) has pointed out that, owing to irregularities in chromosomal distribution, a germ cell might be formed containing two chromosomes of one pair and lacking both representatives of another pair. The number of chromosomes would therefore remain constant, he states, but such germ-cells would be entirely deficient in a particular kind of chromosome. He has further shown ('15*a*, p. 298) that if both members of one pair of chromosomes may pass to one pole of the heterotypic spindle, resulting in a 6–8 distribution of chromosomes, it is conceivable that both members of another pair might, on rare occasions, pass to the opposite poles at the same time. This would equip each daughter nucleus with 7 chromosomes, but not with the usual combination, *A B C D E F G*. Let us assume that this has occurred during male reduction and that two pollen grains bearing *a′a′– c′ d′ e′ e′ f′ g′*, and two bearing *– bb′ c′d′e′ f′g′* chromosomes have been formed. Then should one of these male gametes, say of the first type, unite with a regular 7-chromosome female gamete, we should expect the 14-chromosome plant resulting to have *aa′a′ b– cc′ dd′ ee′ ff′ gg′* chromosomes instead of the usual *aa′ bb′ cc′ dd′ ee′ ff′ gg′* combination. Gates and Miss Thomas suggested that "the variability of the *lata-semilata* series may depend upon the fact that the extra chromosome belongs to a different pair in different cases," and add: "since there are seven pairs of chromosomes, we should then expect seven more or less distinct *lata*-like types," but conclude that "there is at present no evidence that the plants having 15 chromosomes can be divided in this way."

If both members of any one of the seven pairs of chromosomes were capable of passing to one pole, while both members of any one of the remaining six were capable of passing to the opposite pole during male, as well as during female, reduction; if regular and irregular 7-chromosome male and female gametes were formed capable of uniting with each other and producing viable seeds, a large number of 14-chromosome combinations would be possible. If the common types of 15-chromosome mutant offspring of *O. Lamarckiana* could be limited to seven, one might assume that these are "half mutants" (borrowing de Vries's term, but applying it differently) resulting in each case from the union of a regular 7- with an 8-chromosome gamete, each of the latter entering into the union differing from every one of the remaining six with respect to the particular extra chromosome which it possesses. The union of any one of the many irregular 7-chromosome gametes with an 8- might produce one of the rarer types of 15-chromosome mutants and such a form might be regarded as a "whole mutant" (de Vries). The objections to these suggestions are obvious: irregular 7-chromosome distributions would be expected to occur more rarely than 6–8, and germ-cells resulting from the former would be expected to unite with regular 7-, producing 14-chromosome half mutants in the vast majority of cases, and to unite with cells having 8 chromosomes only in extremely rare instances. We would be obliged to conclude that the common 14-chromosome mutant offspring of *O. Lamarckiana* result from ♀ or ♂ regular (*Lamarckiana*) 7 + ♀ or ♂ irregular 7. If such were the case, one of these mutants, such as *O. nanella*, for example, could reproduce itself only by means of the union of dissimilar gametes,[19] probably of the same types as those which entered into the original combination. We have designated the *Lamarckiana* combination as $a\ b\ c\ d\ e\ f\ g + a'\ b'\ c'\ d'\ e'\ f'\ g' = aa'\ bb'\ cc'\ dd'\ ee'\ ff'\ gg'$; then if the mutant *nanella* resulted from $a\ b\ c\ d\ e\ f\ g + a'\ b'b' - d'\ e'\ f'\ g' = aa'\ bb'b'\ c-\ dd'\ ee'\ ff'\ gg'$, and if the male and female gametes produced by the mutant were each represented by the two types of gametes entering into the original combination, *O. Lamarckiana* × *O. nanella* should produce two types of offspring: $a\ b\ c\ d\ e\ f\ g + a'\ b'b' - d'\ e'\ f'\ g' = aa'\ bb'b'\ c-\ dd'\ ee'\ ff'\ gg'$, *O. nanella*, and $a\ b\ c\ d\ e\ f\ g + a'\ b'\ c'\ d'\ e'\ f'\ g' = aa'\ bb'\ cc'\ dd'\ ee'\ ff'\ gg'$, *O. Lamarckiana*. The same results should be secured from the reciprocal cross. As a matter of fact, de Vries ('13, p. 207) has shown that these are the results obtained from the two crosses; but how shall we explain the behavior of *O. nanella*, selfed? It is well known that this mutant breeds true, while on the basis of our previous assumptions, we should expect it to produce three types of offspring: (1) $a\ bb-d\ e\ f\ g + a'\ b'b'-d'\ e'\ f'\ g' = aa'\ bbb'b'-dd'\ ee'\ ff'\ gg'$, unlike both parents; (2) $a\ b\ c\ d\ e\ f\ g + a'\ b'b'-d'\ e'\ f'\ g' = aa'\ bb'b'\ c-\ dd'\ ee'\ ff'\ gg'$, *O.*

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[19] Unless apogamous development were possible.

nanella. The reverse combination should also reproduce the parental type. (3) $a\ b\ c\ d\ e\ f\ g + a'\ b'\ c'\ d'\ e'\ f'\ g' = aa'\ bb'\ cc'\ ee'\ ff'\ gg'$, *O. Lamarckiana.* The first combination might be excused on the pretext of incompatibility, but this would hardly be sufficient to account for the absence of *O. Lamarckiana* from among the offspring of selfed *nanella.* Our difficulties are not lessened, as a little figuring will show, by assuming that two types of gametes are produced by one sex, and only one by the other, such as ♀ $a\ bb-d\ e\ f\ g$ and $a\ b\ c\ d\ e\ f\ g$ + ♂ $a'\ b'b'-d'\ e'\ f'\ g'$ or ♂ $a'\ b'\ c'\ d'\ e'\ f'\ g'$; or by assuming that all of the female gametes are of one type and all of the male of another type. Let us then consider the problem from another viewpoint. We may assume that these irregular 7-chromosome mutant gametes of *O. Lamarckiana*, notwithstanding their numerous opportunities to unite with regular (*Lamarckiana*) 7-, are incapable of doing so, because of incompatibility, and that a gamete of this type can unite only with another of its kind: $a\ bb - d\ e\ f\ g + a'\ b'b' - d'\ e'\ f'\ g' = aa'\ bbb'b' - dd'\ ee'\ ff'\ gg'$, *O. nanella;* also that the mutant produces male and female gametes of the same, single type. Our difficulties would still be with us, for *O. nanella* × *O. Lamarckiana*, and the reciprocal, would result in a new type (the same in both instances) quite unlike either parent. Furthermore, if the original irregular 7-chromosome mutant gamete produced by *O. Lamarckiana* were incapable of uniting with a regular 7-*Lamarckiana* gamete in *O. Lamarckiana* selfed, we would expect the two to be unable to unite in the crosses, yet we know that seed and offspring are readily secured from both. Even should we assume that the *nanella* group $aa'\ bbb'b' -dd'\ ee'\ ff'\ gg'$, resulting from the union of identical gametes, produces female gametes of one type and male of another, such as $a\ bbb-d-f\ g$ and $a'\ b' - d'\ e'e'\ f'\ g'$, our difficulties would not disappear. It seems impossible, on a chromosomal basis, to find an explanation for the fact that *nanella* and *Lamarckiana*, when selfed, produce only *nanella* in the first case, and only *Lamarckiana* in the second (barring rare exceptions), but that *O. nanella* × *O. Lamarckiana* and the reciprocal, produce both parental types in each case. Truly he who attempts to explain mutation on a strictly chromosomal basis finds his pathway beset with many obstacles.

We do not know whether 15-chromosome mutant offspring of 14-chromosome *O. Lamarckiana* result from unions of ♀7 with ♂8, or ♀8 with ♂7, or from both combinations; since there is considerable evidence to indicate that functional 8-chromosome cells of one sex only are produced by certain 15-chromosome individuals, and that these, in many forms, are female, it is possible that the functional 8-, perhaps even all functional 7+-chromosome germ-cells produced by *O. Lamarckiana* and certain 14-chromosome mutant derivatives, are female.[20] More attention has been directed to the study of male than female reduction in various forms. Gates ('10, reported in 1907) and Davis ('11) each recorded 6–8 distributions of male heterotypic chromosomes in *O. Lamarckiana*, but as Davis remarks (p. 952) "we do not know whether or not fertile pollen-grains may be formed with chromosomes in a greater or less number than the normal."[21] Gates and Miss Thomas ('14) found the expected 7–8 distributions of heterotypic chromosomes in the pollen mother cells of *O. lata;* we know that 8-chromosome female gametes are produced by this form, yet we shall see that it appears that very few, if any, 8-chromosome male gametes, capable of functioning, are formed; nor is the presence of the two 16-chromosome plants in the C.S.H. culture of *O. Lamarckiana* positive proof of the production of both male and female 8-chromosome germ-cells, since we do not know whether these individuals arose from 8 + 8, or 9 + 7 unions. It is quite certain, however, that 7+-chromosome cells of one sex or the other, if not of both, are formed occasionally, since 15-chromosome mutants are quite common.

We may summarize our conclusions, therefore, as follows:

(*a*) *15-chromosome mutant offspring of 14-chromosome forms are not invariably distinguished by the somatic characters of* O. lata, O. semilata *or* lata-*like forms.*

(*b*) Lata-*like forms, and those combining certain* lata *characters with others not distinctive of* O. lata, *are not invariably characterized by 15 chromosomes.*

Thus far we have considered only (*a*) whether when a meiotic irregularity in a 14-chromosome form results in the production of a

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[20] This statement merely expresses a possibility and not the writer's established convictions. If 7+-chromosome male gametes, capable of functioning, are never produced by *O. Lamarckiana*, then we must concede that *O. gigas* de Vries arose in some one of the various ways suggested by Gates (and recent evidence tends to strengthen, rather than weaken, Gates's arguments in support of this conclusion) and that it was not the product of the union of two 14-chromosome gametes, as maintained by Stomps and Lutz.

[21] By *O. Lamarckiana*.

15-chromosome offspring, such an individual will have the leaves and habit of *O. lata* or *O. semilata* invariably, or even in the majority of cases (Gates, '13) and (*b*) whether the frequency of the occurrence of an irregular distribution of the chromosomes of 14-chromosome plants into 6–8 groups may determine the frequency with which *lata*-like mutants will appear (Gates and Miss Thomas, '14). We have yet to consider (*c*) whether when *O. lata* is crossed with its 14-chromosome parent, or is selfed, the percentage in which *O. lata* appears among the offspring is indicative of the number of 9- and 7-chromosome germ-cells which function (Gates and Miss Thomas). This question will be treated under the following head.

3. *Are 15-chromosome Forms Inconstant?*

Of the twenty-one 15-chromosome mutants which Gates and Miss Thomas reported, three were identified as *O. semilata*, one as *lata* to *semilata* and two as *semilata* to *lata*. Referring to de Vries's cultures of *O. lata* and *O. semilata* they say (p. 527): "*Oe. lata* was classed by him as an inconstant species, but *semilata* was incorrectly classed as constant. They are both obviously inconstant, however, and *the presence of the odd chromosome shows why this must be so*." (Italics not employed in the original.) Gates ('15*a*, pp. 111–112) has since found that the mutant which he described as *semilata* is not the same as de Vries's mutant of this name, but has decided to retain the name for the form reported by Gates and Miss Thomas, since the Amsterdam type is extinct. Therefore, when it becomes necessary to distinguish between these two types, we shall designate them as *O. semilata* de Vries and *O. semilata* Gates, respectively.

Gates and Miss Thomas's statement raises the question, Does the presence of the odd or extra chromosome necessarily render a form inconstant? Are 15-chromosome forms never constant?

Since 15-chromosome forms produce, as a rule, no pollen, very little, or a moderate amount containing a high percentage of bad grains;[22] since seeds are obtained from selfed forms with difficulty, and when secured, usually a much lower percentage of these than of the seeds derived from 14-chromosome forms succeed in germinating in the short time commonly allowed them, their constancy has not been tested on an extensive scale. Inasmuch as we know that in certain cases but one small culture of offspring from these plants, selfed, has been grown, and have reason to believe that only one or two, containing but very few offspring, have been grown from others, and since it is probable that the offspring derived from these forms, as recorded by various workers, do not represent the whole of the possible progeny in any case, *i. e.*, do not represent all that would have been obtained had means been employed to secure the germination of every seed capable of germinating—it is clear that the evidence upon which we would like to base our conclusions is not wholly reliable. This fact should be borne in mind throughout the discussions which follow. Nevertheless, the evidence as it now stands is not devoid of significance.

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[22] Plants having more than 14, but fewer than 28, chromosomes are much more inclined to be male- than female-sterile. Just why this is so, is not yet clear.

O. lata (15) produces, as we shall see, *O. Lamarckiana*, *O. lata*[23] and a certain percentage of mutants,—the number of Lamarckianas greatly exceeding the number of latas in the cultures grown by MacDougal and de Vries. The behavior of *sublinearis*, if a 15-chromosome form, appears to be similar to that of *O. lata*, since the 31 offspring which de Vries obtained from *sublinearis*, selfed ('09, Vol. I., p. 401) were classified by him as follows: 19 *Lamarckiana*, 3 *sublinearis*, 1 *lata*, 1 *nanella*, 1 *albida*, 3 *subovata*, 2 *oblonga* and 1 *gigas*.[24] Here again we see that the number of Lamarckianas greatly exceeded the number of forms which reproduced the characters of the mutant parent. *O. bipartita* (15), selfed, produced *O. Lamarckiana*, *O. bipartita*, a few forms resembling the parent in most ways but having fewer flowers with cleft petals and extra-lobed stigmas than is common, and a few mutants. Here, also, a higher percentage of Lamarckianas than of bipartitas was obtained in the time allowed for the germination of the seeds, probably about 4 months.

As previously stated, the number of chromosomes present in *O. scintillans* is unknown, but it is probable that it is 15. This form, when selfed, according to de Vries ('13, p. 257), produces a variable number of *scintillans;* sometimes 35–40 percent or less and again as high as

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[23] Bartlett ('15*a*, p. 103) calls attention to the similarity in the behavior of *O. lata* and *O. stenomeres* mut. *lasiopetala*. From the latter form, selfed, he obtained 60 percent *stenomeres* and 40 percent *lasiopetala*. Mr. Arzberger has counted 14 chromosomes in *O. stenomeres*; the chromosome number of *O. lasiopetala* has not yet been announced, but it is probably 15.

[24] De Vries has since concluded ('12, p. 34) that this plant and the other identified as *O. gigas* in 1899 ('09, Vol. I., p. 327) were probably triploid, and not tetraploid, forms.

70–80 percent of the total number of offspring reproduce the characters of the mutant parent. The remainder are, for the most part, *O. Lamarckiana*, but with a considerable number of *O. oblonga* ("oft bis 20%") and a few other mutants.

O. semilata Gates (15), is an inconstant form, as Gates and Miss Thomas ('14, p. 532) and Gates ('15*a*, pp. 114–115) have shown, producing *O. Lamarckiana*, *O. semilata* Gates, a few *O. lata* which may be classed as mutants and (p. 114) others "forming a continuous series running to *Lamarckiana*."

O. elliptica, having 15(?), chromosomes, reverts almost entirely to *Lamarckiana*, according to de Vries ('09, Vol. I., pp. 397–398). From one 1895 mutant, selfed, he obtained "some hundred of seedlings," all of which proved to be ordinary *Lamarckiana*. From a second mutant of the same year 500 offspring were secured, 1 of which was *elliptica*, and the remainder *Lamarckiana*. A third 1895 mutant "gave rise to 27 seedlings not one of which was an *elliptica*." From an 1896 mutant he obtained 32 offspring, 5 of which were *elliptica* and the remainder *Lamarckiana;* from an 1899 mutant he secured about 100 offspring, all of which were *O. Lamarckiana*.

O. lata rubricalyx, in which Gates and Miss Thomas counted 15 chromosomes, when selfed, according to Gates ('15*a*, p. 288), produced a nearly uniform lot of offspring (44 plants), "all having the red pigmentation of *rubricalyx*, but were intermediate between *rubricalyx* and *grandiflora* in foliage and buds. . . . The plants which were examined had 14 chromosomes, as was doubtless the case with all of them." No *lata rubricalyx* plants were found among the offspring.

While all of the above forms are clearly inconstant, de Vries's researches indicate that a 15-chromosome form may breed perfectly true. He selected 5 biennial *albida* plants ('09, Vol. I, p. 229) in 1897 and grew a second generation consisting of 86 individuals in 1898 and a third, consisting of 36, in 1899. "Both generations," he adds, "were absolutely constant and exhibited no signs of reversion."

If *O. oblonga* be a 15-chromosome form, it indicates even more strongly (because of the larger number of offspring obtained) that a 15-chromosome form may be constant. During a period of over 13 years, de Vries (pp. 346–348; also, '13, p. 315) selfed a number of *oblonga* mutants and obtained a total of 2,919 offspring, all of which, with the exception of 11 mutants (7 *rubrinervis*, 3 *albida*, 1 *elliptica*) were *oblonga*.

Certain somatic characters of many mutant offspring of *O. Lamarckiana* × *O. Lamarckiana* and of *O. Lamarckiana*, selfed, indicate that *a very large percentage of the mutant offspring of* O. Lamarckiana *have 15 chromosomes and that a larger number of 15- than of 14-chromosome mutant offspring are produced by this form. Not only does there appear to be a larger number of distinct types of 15- than of 14-chromosome mutants, but a higher percentage of 15- than of 14-chromosome mutant individuals produced by* O. Lamarckiana. Many of the mutant offspring of *O. Lamarckiana* never have been brought to flower; furthermore, new forms are appearing each year. It will be necessary to determine the somatic chromosome numbers of a large percentage of the mutant types produced by *O. Lamarckiana*, to bring the 15-chromosome forms to flower, to self flowers on all parts of the plants, to adopt methods which will secure the germination of all viable seeds, and to grow large numbers of offspring,—in order to ascertain whether 15-chromosome forms are more commonly inconstant than constant. The majority of the 15-chromosome forms whose constancy we have considered have produced very few offspring, yet we may safely assert that the evidence available at present indicates that *most 14-chromosome forms are constant and most 15-chromosome forms inconstant.* Furthermore, although our present knowledge of the behavior of 14+-chromosome forms is very limited, largely owing to the infrequency with which good pollen is produced by such forms, it may be stated that the evidence available at present indicates that *inconstancy is commonly associated with the 14+-chromosome condition.* It seems, however, that forms having twice 14 chromosomes are more likely to be constant—in the same sense that *O. gigas* de Vries is constant—than those having more than 14, but fewer than 28, chromosomes.

4. *Factors Determining the Constancy or Inconstancy of 15-Chromosome Forms.*

De Vries obtained the same results from *O. scintillans* selfed, as from *O. scintillans* × *O. Lamarckiana* (pp. 257–262); also the same results from selfed hybrid *lata*, descended through *O. lata* × *O. Lamarckiana* from *O. lata* × *O. semilata*, as from *O. lata* × *O. Lamarckiana* ('09, Vol. I., pp. 240, 360; '13, pp. 244–257). This led him to conclude that female gametes of *O. scintillans* and this hybrid *lata* do not bear the same hereditary characters as the male gametes of these forms; that the characters of the mutant, in the first case, and of the hybrid (which are the same as those of mutant *O. Lamarckiana lata*), in the second, are transferred to the offspring through the egg cells, and not through the pollen; that the pollen, in each case, behaves precisely as the pollen of pure *Lamarckiana* (pp. 257, 258, 262, 272, 273, 323).

At the time of the publication of "Gruppenweise Artbildung", *O. lata* was popularly supposed to be the only 15-chromosome mutant produced by *O. Lamarckiana* or other forms, hence de Vries has discussed these very important results without reference to the chromosome numbers of the plants in question. However, since a number of forms are now known to have 15 chromosomes, their behavior may be further considered in the light of this fact.

Bartlett ('15*a*, p. 103), discussing the behavior of selfed *O. lata* and *O. stenomeres* mut. *lasiopetala*, concludes, in agreement with de Vries, that "it appears that the good pollen grains of *Oe. lata* are genetically the same as those of *Oe. Lamarckiana*, and do not carry the *lata*-characters." "Thus," he states, "it appears that there is a class of mutations of which the eggs are of two kinds; one kind carries the characters of the parent species, the other kind the characters of the mutation. The pollen grains, however, appear to be of one kind only, and to carry the characters of the parent species." He says "we must assume that the male 8-gametes are eliminated" and asks if it is not possible "that the male gametes which carry the characters of the mutation are eliminated because of some physiological defect?" "*Oe. lata*," he states, "produces two classes of gametes, with 8 and 7 chromosomes, respectively. If two 7-gametes fuse, we have *Oe. Lamarckiana;* if a 7-gamete (presumably male) fuses with an 8-gamete (presumably always female) we have *Oe. lata*." We shall see that the evidence indicates such are the usual, though not the invariable, results.

MacDougal ('07) obtained 94 offspring from selfed *O. lata* which were identified as follows: 10 *O. lata*, 80 *O. Lamarckiana*, 1 *O. albida* and 3 *O. oblonga*. *Albida* has 15 chromosomes and *oblonga* 14 or 15. De Vries ('13, p. 256) obtained 442 offspring from a hybrid *lata*, selfed, 33 percent of which were *O. lata* and 4 percent mutants. The remainder were, doubtless, *O. Lamarckiana*. It is probable that several types of 15-, and one or more 14-chromosome forms were included among these 17 or 18 mutants.

In 1908 3 mutant *lata* offspring of *O. Lamarckiana* were selfed at Cold Spring Harbor. A total of 360 seeds were obtained and these were planted at spaced intervals in pans of sterilized soil, December 11 of the same year. 129 germinations resulted; 2 plants died unidentified as seedlings and a third, identified as *O. Lamarckiana*, died later. The remaining 126 were transferred to the garden May 12, 1909. One *lata* from each of the three 1909 cultures was then selfed and the 259 seeds obtained were sown in the same manner as those of the preceding season, March 7, 1910. 99 germinations resulted; all of the seedlings survived and all of the young plants were transferred to the experimental garden May 16, following.

Of these 226 plants, 109 (approximately 50 percent) were clearly identified as *O. lata*, 8 as *O. lata* (?), 57 as *O. Lamarckiana* (approximately 25 percent) and 4 as *O. Lamarckiana* (?). The chromosome numbers of the plants in the second and fourth groups are unknown. In addition to the foregoing there were 7 distinct types of 15-chromosome mutants (23 individuals) which could not be classified either as *O. lata* or as *lata*-like forms. Still other mutant types, whose chromosome numbers were unknown, were believed to be 15-chromosome forms. In addition to the 57 Lamarckianas there were 3 types (4 individuals) of 14-chromosome mutants, quite unlike *O. Lamarckiana*. These are believed to represent approximately the total number of 14-chromosome forms produced. 196 of the 226 plants grown represented types whose chromosome numbers are now known, and but one of the 196 had 16 chromosomes.[25] De Vries's mutants were not classified, but it is quite clear that no one of the 94 offspring which MacDougal obtained from selfed *lata* had 16 chromosomes. We do not know how many of MacDougal's and de Vries's seeds failed to germinate, but we have seen that 63 percent of the Cold Spring Harbor seeds sown failed to germinate in the few months allowed them, hence we do not know what would have been the relative percentages of 14-, 15- and 16-chromosome forms, had all the viable seeds sown germinated.

In connection with these studies of selfed latas, the results obtained from crossing one of these 1908 mutants with *O. Lamarckiana* will be of interest. In 1908 I pollinated *O. lata*, mutant No. 3500, with *O. Lamarckiana*, No. 3814, and covered the stigmas of the latter plant with small quantities of pollen obtained from the former. 15 chromosomes were counted in the somatic cells of No. 3500 and 14 in those of No. 3814. 320 seeds from *O. lata* × *O. Lamarckiana* were planted at spaced intervals in seed pans, December 12, 1908; 49 of this number germinated previous to the time of transplanting in May. Four of the young plants died as seedlings and the remainder were classified as follows:

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[25] In addition to the 14-, 15- and 16-chromosome offspring referred to, one 21- and one 22-chromosome mutant were produced, as previously reported (Lutz, '12). The 226 offspring of these six selfed latas will be carefully tabulated and fully described in a later report.

TABLE II

O. lata No. 3500 × *O. Lamarckiana* No. 3814

	O. Lamarckiana	*O. aberrans*	*O. lata*	*O. albida*	Type 5432	Totals
14 chromosomes......	15	....	....	....	....	15
14^{+1} "	....	1	....	....	....	1
15 "	....	....	27	1	....	28
? "	....	....	....	....	1	1

On December 11, 1908, the same number of seeds from the second cross (*O. Lamarckiana* × *O. lata*) were planted in the same manner as the above. Only 18 germinated; therefore, on February 1, 1909, 119 seeds from the same capsules as the preceding were planted and 58 seedlings obtained previous to the middle of May. The 76 plants derived from this cross were classified as follows:

TABLE III

O. Lamarckiana No. 3814 × *O. lata* No. 3500

	O. Lamarckiana	*O. nanella*	Type 3514 (modified *rubrinervis?*)	*O. lata*	*O. bipartita* (?)[26]	Totals
14 chromosomes......	63	1	9	....	....	73
15 "	....	....	....	1	2	3

From these tables we see that in the time allowed for germination, almost twice as many 15- as 14-chromosome offspring were derived from *O. lata* × *O. Lamarckiana*, while only 3, possibly only 1, of the 76 plants derived from *O. Lamarckiana* × *O. lata* had 15 chromosomes. Since one or more 15-chromosome mutants usually are found in *Lamarckiana* cultures of this size, it is probable that the 15-chromosome offspring of *O. Lamarckiana* × *O. lata* resulted from ♀8 + ♂7 and possible (providing 8-chromosome gametes are formed by *O. Lamarckiana*) that one or two of those derived from the reciprocal cross were products of ♀7 + ♂8 unions. Notwithstanding the fact that 85 percent of the *lata* × *Lamarckiana*, and 83 percent of the *Lamarckiana* × *lata*, seeds failed to germinate in the time allowed them—approximately the same percentage of failures in both cases—15-chromosome forms appeared in considerable numbers among the offspring of the first cross, but were almost entirely absent from the second culture. However, we do not know what the percentages of 14- and 15-chromosome offspring would have been had all the seeds of each cross germinated.

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[26] The identification of these supposed bipartitas was based upon the characters of the greenhouse rosettes, as the plants were not transferred to the garden.

Bearing in mind that we do not know "what may be the changes of front when exact data become available," it may be said that the C.S.H. cultures of mutant *O. Lamarckiana lata* selfed and crossed both ways with *O. Lamarckiana* confirm, in the main, the earlier statements by de Vries and Bartlett and point to the following conclusions regarding this mutant: (*a*) *Lata* characters are transmitted through a portion of the egg cells, and not, except possibly in rare instances, through the pollen of this form. (*b*) 8- and 7-chromosome female gametes, capable of functioning, but as a rule, only 7-chromosome male gametes, capable of functioning, are produced by *O. lata*.[27] The majority of the 8-chromosome female gametes (probably not all) are bearers of *lata* characters, while the majority of the male and female 7-chromosome gametes (probably not all) are bearers of *Lamarckiana* characters. (*c*) It now appears that when offspring result from 8 + 7 and 7 + 7 unions, the majority of the former have *lata*, or *lata*-like characters, and the majority of the latter *Lamarckiana* characters, but, as in the case of *O. Lamarckiana*, selfed, it is not safe to assume that such are the invariable results, since it is probable that at least a portion of the 15- and 14-chromosome offspring derived from *O. lata* selfed, *O. Lamarckiana*, selfed, and *O. lata* × *O. Lamarckiana* which cannot be classified as *O. lata* and *O. Lamarckiana*, are products of 8 + 7 and 7 + 7 unions, respectively. As in the case of *O. Lamarckiana*, *O. albida* is one of the common mutant offspring of *O. lata*, selfed, and of *O. lata* × *O. Lamarckiana* and it seems quite probable that this form results from 8 + 7 unions.

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[27] Fourteen- and fifteen-chromosome mutants, particularly the latter, are found in practically all fair-sized cultures of *O. lata* × *O. Lamarckiana*. No statement can be made concerning the appearance of these forms in cultures of the reciprocal cross, since only one has been reported thus far.

While, as we have seen, de Vries has shown that *O. scintillans*, when selfed or pollinated by *O. Lamarckiana*, behaves in much the same way as *O. lata* under similar conditions, statements concerning the chromosomal combinations resulting from these operations must be wholly speculative since the numbers of chromosomes present in *O. scintillans* and its *oblonga* offspring are unknown. However, if *scintillans* has 15, as is probable, there is much evidence to indicate that 7- and 8-chromosome female gametes, capable of functioning, and, as a rule, only 7-chromosome male gametes, capable of functioning, are produced by this form,[28] whether *oblonga* has 14 or 15 chromosomes. That only 7-chromosome male gametes are produced which behave in every way like the 7-chromosome male gametes of *O. Lamarckiana* is clearly indicated by the fact that while *O. scintillans*, selfed, and *O. scintillans* × *O. Lamarckiana* produce *O. scintillans*, *O. Lamarckiana* and *O. oblonga*, *O. Lamarckiana* × *O. scintillans* yields 100 percent *O. Lamarckiana* (de Vries, '13). A noteworthy peculiarity in the behavior of *O. scintillans* is the relatively large number of offspring of one type derived from the mutant, selfed, and pollinated by *O. Lamarckiana*, which display neither the characters of *O. scintillans* nor of *O. Lamarckiana*, but of the mutant, *O. oblonga*. This indicates either that a relatively large percentage of the offspring resulting from 8 + 7 unions fail to reproduce the characters of the mutant parent, or that a relatively large percentage of those derived from 7 + 7 unions fail to display the characters of *O. Lamarckiana*.

The behavior of *O. bipartita* and of *O. sublinearis* (if the latter has 15 chromosomes, and it is probable that it has) indicates that all, or nearly all, of the gametes of one sex which are capable of functioning, contain 7 chromosomes, while a portion of those of the other sex contain 7 and the remainder 8. The same may be said of *O. semilata* Gates if the offspring which Gates refers to as "forming a continuous series running to *Lamarckiana*" have either 14 or 15 chromosomes.[29]

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[28] In the case of *O. lata* and other forms to be discussed in this report, it will be understood that the writer does not exclude the possibility of other gametes being formed occasionally in addition to those enumerated—gametes having fewer than 7, or more than 7 or 8 chromosomes. For instance, as earlier stated, it is conceivable that 6-chromosome gametes may function in union with 8- or 8+-. It is possible that 9-chromosome gametes, capable of functioning, may be produced occasionally by 14- and 15-chromosome forms, particularly the latter, and we know that there is much evidence to show that 14-chromosome gametes are sometimes produced by 14-chromosome forms (possibly also by 15-) and 15-chromosome gametes by 15-chromosome forms.

O. stenomeres mut. *lasiopetala*, as Bartlett ('15*a*) has pointed out, resembles *O. Lamarckiana* mut. *lata* in its behavior, with, of course, this exception: the 14-chromosome offspring of the former bear the characters of *O. stenomeres*, and not of *O. Lamarckiana.* In each case, however, the offspring bear the characters of the 14-chromosome form which produced their mutant parent. Furthermore, as in the case of *O. lata*, a portion of the offspring (presumably having 15 chromosomes) reproduce the characters of the mutant parent.

The behavior of *O. elliptica*, if a 15-chromosome form, indicates that all of the gametes of one sex, capable of functioning, have 7 chromosomes, while the majority of those of the other sex which are capable of functioning have 7 chromosomes, and only a very few, 8.

The behavior of *O. nanella lata* indicates that only 7-chromosome female gametes are produced by this form since de Vries ('09, Vol. I., p. 374) found that *O. nanella lata* × *O. nanella* "gave rise to ordinary *nanella* only." From *O. lata* × *O. Lamarckiana* de Vries ('13, p. 257) obtained two dwarfs through mutation. "Der eine hatte nebenbei die Merkmale der *Lamarckiana*, der andere diejenigen der *Lata*. Beide hatten Pollen, und wurden damit rein befruchtet. . . . Die letztere gab zwar auch nur Zwerge . . . , spaltete sich aber in bezug auf die *Lata*-Merkmale in 9 *Lata*-Zwerge und 18 gewöhnliche Zwerge, . . . ". The latter, upon self-fertilization, proved constant, but the *lata* dwarfs behaved in the same manner as the parent, when selfed.[30] If this *lata* dwarf is the same form as de Vries's *O. nanella lata*, then the available evidence indicates that all of the female cells of this mutant which are capable of functioning contain 7 chromosomes, while the majority of the male contain 7 and a smaller number 8.[31] Little or no consideration should be given to this evidence, however, since we do not know that the two mutants combining *nanella-lata* characters were duplicate types; furthermore, we have no assurance that a sufficient number of offspring of *O. nanella lata* × *O. nanella* were grown to prove that *O. nanella* only, invariably results from this cross; and finally, since only 27 offspring were derived from the later mutant, selfed, it is clear that we are not justified in formulating definite conclusions concerning its behavior.

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[29] This condition was earlier indicated by Bartlett ('15*a*, p. 103) in the statement that "*Oe. scintillans* acts like *Oe. lata* in every way."

[30] *O. rubrinervis lata*, which appeared in Schouten's 1906 culture of *O. rubrinervis* (Schouten, '08), suggests a 15-chromosome condition, as Gates ('15) has stated, but since the chromosome number of this plant is unknown and that of the parental type unestablished, the behavior of this mutant will not be discussed at present.

[31] The behavior of *O. oblonga*, if a 15-chromosome form, is somewhat contradictory and will not be discussed here.

The behavior of *O. lata rubricalyx* is somewhat unique. If all of the offspring of the mutant, selfed, had 14 chromosomes, as Gates thinks probable, this fact would indicate that all of the male and female cells produced which were capable of functioning, had 7 chromosomes. This is further indicated by the fact that although Gates crossed the mutant both ways with several other forms, "the offspring (few in number) which developed proved to be all of 14-chromosome types." He does not state whether the several forms employed in these crosses were 14- or 15-chromosome plants, but the results indicate that only ♀7 + ♂7 unions occurred in every case, or at least that only seeds resulting from these unions germinated in the time allowed them. Of especial interest is the fact that the 14-chromosome offspring of this plant were intermediate between *rubricalyx* and *grandiflora* (the grandparents). Would the mutant behave differently if produced by *rubricalyx*, selfed? Is this precise type ever produced by *rubricalyx?*

In the case of *O. albida*, all of the cells of one sex appear to contain 7 chromosomes and all of the other 8, since de Vries obtained *albida* offspring only from this form, selfed.

Thus, in addition to *O. lata*, the records show that a certain number—commonly more than half—of the offspring derived from selfed *O. bipartita*, *O. scintillans*, *O. sublinearis* and almost all of those obtained from selfed *elliptica* (the evidence is not clear in regard to *O. semilata* Gates) were 14-chromosome plants and that all of them, or all but a few mutants (providing the *oblonga* offspring of *O. scintillans* had 15 chromosomes), were *O. Lamarckiana*.[32] Hence the evidence as it now stands indicates that all but relatively few of the 7-chromosome male and female gametes of these plants (providing *scintillans* and *sublinearis* are 15-chromosome forms) are bearers of *Lamarckiana* characters[33] and that the mutant characters of the 15-chromosome parent in each case are transmitted to the offspring through the 8-chromosome gametes. In cases with which we are familiar, the 8-chromosome cells appear to be produced wholly, or almost wholly, by one of the two sexes; this is clearly female in certain cases, but we may find that these cells are male in others.[34] Without considering the question of whether these forms, when given off as *Lamarckiana* mutants are "half mutants" (de Vries), it is clear that a *Lamarckiana* offspring is produced by selfed 15-chromosome mutant derivatives of *O. Lamarckiana* when two gametes, each bearing *Lamarckiana* characters, and, possibly, the chromosomal combination peculiar to these gametes, unite and produce a seed capable of germinating. Bartlett ('15*a*), after stating that certain mutants appear to produce two kinds of eggs, one carrying the characters of the mutant producing them and the other the characters of the parent species, while the pollen grains appear to be of one kind only and to carry the characters of the parent species, adds: "If so, *Oe. lata* might be supposed to originate by the union of a *lata*-egg (itself constituting the true mutation) with a *Lamarckiana*-sperm." Accepting Bartlett's suggestion, it may be said of all 15-chromosome mutant derivatives of *O. Lamarckiana* which, when selfed, produce two types of offspring, one duplicating the characters of the mutant parent and the other those of *O. Lamarckiana*, that an offspring of the first type is obtained from these forms, selfed, when an 8-chromosome gamete bearing the characters of the mutant parent, and, possibly, the chromosomal combination peculiar to these gametes, unites with a 7-chromosome gamete bearing *Lamarckiana* characters, and possibly, the chromosomal combination peculiar to *Lamarckiana* gametes, and produces a seed capable of germinating. If we consider this matter on a strictly chromosomal basis, we will concede, of course, that 9 + 6, or any other union which produces the same chromosomal combination as the ordinary 8 + 7, might result in an offspring which would duplicate the vegetative characters of the 15-chromosome parent. However, *since 14-chromosome offspring of 15-chromosome plants and 15-chromosome offspring of 14-*

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[32] It is probable that the list of forms which produce large numbers of *O. Lamarckiana* when selfed, might be extended to include many other direct and indirect 15-chromosome mutant derivatives of *O. Lamarckiana*, but not all; *O. nanella lata* and *O. rubrinervis lata* may be suggested, among others, as probable exceptions.

[33] The evidence indicates that all of the 7-chromosome gametes of one sex produced by *O. elliptica* and all but comparatively few of the opposite sex, which are

capable of functioning, are bearers of *Lamarckiana* characters. It is probable that 7-chromosome *Lamarckiana* gametes (of one sex) are produced by *O. albida*, but experimental evidence has not indicated the facts in the case.

[34] Doubtless these assertions, and the conclusions regarding *O. lata* under the heads of (*a*), (*b*) and (*c*) on page 87 would be equally applicable to many other 15-chromosome mutant derivatives of *O. Lamarckiana*, if the names of these mutants, in the latter case, were substituted for that of *O. lata*.

chromosome plants never duplicate the vegetative characters of their parents, it is clear that 8 + 8 (lata *8 +* lata *8, for example) could not produce an individual having the same vegetative characters as the 15-chromosome parent.*[35]

In view of the fact that when certain 15-chromosome forms are selfed, the parental mutant type appears to be reproduced by 8 + 7, and never by 8 + 8, unions, one may ask whether we are not justified in asserting that the 7-chromosome cells are as truly transmitters of the mutant characters as the 8-; it seems that they are not, for the 22-chromosome offspring of *O. lata* × *O. gigas*, which presumably result from ♀ 8 + ♂ 14 unions, suggest *lata-gigas* characters, while 21-chromosome hybrids, which doubtless result from ♀ 7 + ♂ 14 unions, suggest *Lamarckiana-gigas* combinations. Since the latter bear no trace of *lata* characters, it is clear that these are transmitted through the 8-, and not through the 7-chromosome gametes.

If 7- and 8-chromosome male and female gametes were produced in equal numbers, all capable of functioning in union with 7- and 8-chromosome cells of the opposite sex, we would expect a selfed 15-chromosome form to produce 14-, 15- and 16-chromosome offspring in the ratio of 1 : 2 : 1. How, then, shall we explain the fact that the number of 14-chromosome offspring produced usually (not invariably, as we have seen) exceeds the number of 15-, while 16-chromosome forms are almost unknown in such cultures? How shall we explain the fact that F_1 cultures derived from 15-chromosome forms pollinated by 14- usually contain many more 14- than 15-chromosome plants?[36]

1. In the first place, it will be recalled that no evidence has been brought forward to show that 8-chromosome male gametes, capable

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[35] It is quite probable, however, that *lata*-like mutants may result from *lata* 8 + *lata* 8.

[36] After the manuscript for this report had left the writer's hands, an important contribution from de Vries appeared, entitled "New dimorphic mutants of the Oenotheras" (Bot. Gaz. **62**: 249–280, Oct., 1916). In this report de Vries has shown that *O. cana*, which is probably a 15-chromosome form, produces *O. cana* and *O. Lamarckiana* when selfed, but that a larger percentage of the offspring duplicate the characters of the mutant parent when a biennial, than when an annual, plant is employed. This fact demonstrates, as he states, that the behavior of *O. cana* is largely dependent upon the vigor of the individual employed. De Vries believes that this is true of other dimorphic mutants, since he had earlier demonstrated this difference in the behavior of annual and biennal *scintillans*. The bearing of these important facts upon the statements which are included under the heads of 1, 2 and 3 above, will be discussed in a later publication.

of functioning, are produced by 15-chromosome forms. So far as has been observed, the male gametes appear to be, with rare exceptions, exclusively 7-. It is possible that the embryo-sac is more frequently differentiated from a 7- than from an 8-chromosome group, and that more 7- than 8-chromosome eggs, capable of functioning, are produced.[37]

2. Since many 14+-chromosome forms produce both 7- and 7+-chromosome female gametes (♀ *lata* 7 and 8), but are usually male-sterile, hence produce, ordinarily, neither 7- nor 7+-chromosome male gametes, our knowledge of controlling factors is still too limited to warrant the suggestion that 7+-chromosome groups are more likely than 7- to fail to produce male gametes capable of functioning.[38] It is probable, however, that 7-chromosome gametes unite with 7- and produce seeds capable of germinating more readily than any other combination. There is also considerable evidence to indicate that, as a rule, gametes unite and produce viable seeds more readily (*a*) when one gamete contains 7, and the other a multiple of 7, chromosomes[39] (7 + 14; 21-chromosome *Lamarckiana-gigas* offspring of *O. lata* × *O. gigas*, *O. Lamarckiana* × *O. gigas*, etc.); (*b*) when both gametes contain a multiple of 7 chromosomes (14 + 14; 28-chromosome offspring of *O. gigas*, selfed, than (*c*) when each contains more than 7, but fewer than 14, chromosomes.[40] Plants having more than 14,

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[37] Gates and Miss Thomas ('14) have stated that 8-chromosome megaspores of *O. lata* evidently have fewer prospects of functioning than 7-chromosome megaspores, since the percentage of latas derived from *lata*×*Lamarckiana* usually falls below 20 percent, and sometimes to 4 percent.

[38] We should not overlook the fact that in absolutely male-sterile 15-chromosome forms, 7-chromosome male gametes are eliminated as completely as the 7+-.

[39] There is also some evidence to indicate that 8- unites with 14- and produces seed capable of germinating more readily than two gametes, both of which contain more than 7, but fewer than 14, chromosomes (22-chromosome offspring of *O. lata* ×*O. gigas*). We have no evidence whatever on which to base conclusions regarding 7 + 13. Whether 8 + 14 combinations are less likely to occur and produce viable seed than 7 + 14, cannot be stated. The culture of 133 offspring which de Vries ('13, p. 186) obtained from *O. lata* × *O. gigas* in 1907 consisted of 65 *Lamarckiana-gigas* (presumably 7 + 14) and 68 *lata-gigas* (presumably 8 + 14), offspring.

[40] The writer's experience with cultures of 14- and 15-chromosome forms pollinated by 28- has not been sufficiently extensive to justify the assertion that seed and offspring are less readily secured from these than from 28- pollinated by 28-, but numerous attempts were made to pollinate 28-chromosome *O. gigas* de Vries with 14- and 15-chromosome forms, invariably with the same result; only flat seeds, or seed-like structures were secured, and these, of course, were utterly incapable of germinating. De Vries and Davis, however, have each grown offspring of *O. gigas* × *O. Lamarckiana*, though Davis's culture ('10) was quite small,—12 plants.

but fewer than 28, chromosomes commonly produce no pollen, very little, or at most, only a moderate amount; furthermore, one ordinarily finds that very few of the grains produced are normal appearing, hence the majority are probably incapable of functioning. On the other hand, 14- and 28-chromosome plants not only produce larger quantities of pollen, as a rule, but usually a much larger percentage of the grains produced are normal appearing. In the case of 14-chromosome forms, usually about 60–70 good appearing grains per 100 (sometimes fewer, sometimes more) are found in the early and mid-season buds. These factors are undoubtedly primarily responsible for the difficulty commonly experienced in obtaining seeds from forms having (14+–28)[41] chromosomes, as compared with the relative ease with which they are ordinarily secured from 14-chromosome forms, selfed, or even from selfed 28-chromosome plants; but it does not tell us why seeds of 21-chromosome forms, when secured, germinate much less readily than seeds of 14-chromosome plants and less readily than those of 28-chromosome *O. gigas* de Vries.

Gates ('15*a*, p. 194) says: "It is clear that triploidy leads to the production of many new chromosome-numbers, through the irregularities it introduces into the meiotic phenomena. . . . It is at present unknown whether the number alone determines the viability, or whether particular chromosome combinations will, owing to incompatibility, fail to produce an embryo after fertilization." Elsewhere (p. 234) he speaks of the difficulty experienced in making "giant crosses" (doubtless referring to crosses of 28- with 14- and 15-chromosome forms) and says: "This is undoubtedly a result of the unbalanced chromosome numbers and the meiotic irregularities to which they lead, ..."

The writer has long thought it probable that the incompatibility of certain combinations, particularly such as those brought about by selfing triploid forms, is partially responsible, not only for the small number of seeds produced, but for the relatively small number of seeds which germinate. Doubtless many of the heterotypic distributions in triploid forms (20- and 22-, as well as 21-chromosome individuals) are irregular, resulting in the production of daughter groups with fewer than 7, more than 7, regular 7, or, possibly, an irregular assortment of 7 chromosomes. Even such combinations as 10 + 10, 10 + 11 and 11 + 11 in selfed 21-chromosome forms may be less compatible than is apparent, for two gametes having the same number of chromosomes, or nearly so, may be represented by entirely different chromosomal combinations. Yet Gates's assertion that "it is at present unknown whether the number alone determines the viability, or whether particular chromosome combinations will, owing to incompatibility, fail to produce an embryo after fertilization" voices a question which is still unanswered. If we believe that each of the 14-chromosome mutant types produced directly by *O. Lamarckiana* is represented by a chromosomal combination differing from that of the parent, then in crossing these various types one would expect to find some of the 7 + 7 combinations resulting less compatible than that of *O. Lamarckiana*, selfed. All 14-chromosome forms which have been tested by the writer have been found to produce an abundance of seed when selfed or crossed with other 14-chromosome forms; furthermore, relatively larger percentages of these seeds (compared with those derived from forms having more than 14, but fewer than 28, chromosomes) were found to be capable of germinating. No evidences of incompatibility have been observed thus far; indeed fertility appears to be associated with the 14-chromosome condition wherever found.

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[41] More than 14, but fewer than 28.

Ordinarily only a small percentage of the few seeds derived from selfed 21-chromosome forms are capable of germinating in the few months commonly allowed them in seed pans. De Vries ('09, Vol. I, p. 261) has shown that *Lamarckiana* seeds may lie in the ground for two or more years before germinating, and the writer has recently verified these results. If so many 14-chromosome seeds may germinate late one may be led to inquire whether certain 14+-chromosome seeds may not be even more inclined to germinate tardily. In the opinion of the writer, the lower percentage of germination commonly exhibited by seeds of most 14+-chromosome forms is due to the total inability of many 14+-chromosome seeds to germinate, rather than to a greatly increased tendency on the part of these seeds to germinate late. It is furthermore probable that, ordinarily, a larger percentage of the products of selfed 14+-, than of selfed 14-chromosome forms are merely seed-like structures, and therefore incapable of germinating. Particularly is this probably true of the products of selfed triploid individuals, for one frequently finds that a large percentage of the few seeds obtained are small, flat and unpromising appearing.

Regarding the relative percentages of 14-, 15- and 16-chromosome offspring derived from selfed 15-chromosome forms, it is possible that gametic incompatibility is partly responsible for the usual production of fewer 15- than 14-chromosome offspring by 15-chromosome forms pollinated by 14-, and by selfed 15-chromosome plants. *Lata* 8 + *Lamarckiana* 7 is plainly an unbalanced combination, but *lata* 8 + *lata* 8, assuming that the two gametes combined duplicate chromosomes ($aa\ b\ c\ d\ e\ f\ g + a'a'\ b'\ c'\ d'\ e'\ f'\ g'$), could be designated as a balanced combination; would these two gametes be compatible? More so than *lata* 8 + *Lamarckiana* 7 ($aa\ b\ c\ d\ e\ f\ g + a'\ b'\ c'\ d'\ e'\ f'\ g'$) and as much so as *Lamarckiana* 7 + *Lamarckiana* 7 ($a\ b\ c\ d\ e\ f\ g + a'\ b'\ c'\ d'\ e'\ f'\ g'$)?

Whatever the facts regarding these questions it is clear that if 8-chromosome gametes, capable of functioning, are produced by one sex only (barring rare exceptions), as appears to be the case in many instances at least, this alone is sufficient to explain the almost complete absence of 16-chromosome mutants in cultures of 15-chromosome forms, selfed.

3. The elimination of the extra chromosome by means of one or more of the various processes observed by Gates and Miss Thomas[42]

[42] Gates ('15*a*, p. 288) commenting upon the fact that *O. lata rubricalyx* produced, presumably, only 14-chromosome offspring when selfed and crossed both ways with several other forms, said: "Since there was an abundance of pollen, it would appear probable that many of the grains must have received the extra chromosome and that the latter was frequently lost during the divisions in the pollen tube." Gates's suggestion is well worth considering, but we should not overlook other possibilities in the case. While Gates and Miss Thomas ('14, p. 545) tell us that *lata rubricalyx* "produced a good amount of viable pollen" and that it "developed long stout capsules" (p. 533), thereby indicating that seeds were produced in abundance, Gates ('15*a*) further states that few offspring were obtained from crosses of this mutant both ways with several other forms. Since 15-chromosome forms commonly produce very little pollen capable of functioning, or none at all, it is probable that these plants were 14-chromosome forms; however that may be, let us assume that only 7-chromosome eggs, capable of functioning, were produced by *lata rubricalyx*. Even though well-filled fruits were developed, if only relatively few of the large number of seeds produced succeeded in germinating, perhaps those resulting from ♀7 + ♂8 unions were incapable of germinating, or failed to germinate in the time allowed them. Perhaps one of the 15 male chromosomes was eliminated occasionally during reduction by one or more of the numerous irregularities observed by Gates and Miss Thomas in this form, such as failure to be included within the heterotypic daughter nucleus and subsequent degeneration; degeneration on the homotypic spindle, etc. In this way many more 7- than 8-chromosome pollen grains may have been formed. *We have no assurance that every seemingly good grain is capable of functioning*; neither can it be said, because 15-chromosome offspring have not been found in cultures of *O. Lamarckiana* pollinated by certain 15-chromosome

during reduction in 15-chromosome forms may result in the production of a greater number of 7- than of 8-chromosome female gametes and may partially account for the greater number of 14- than of 15-chromosome forms among the offspring of these plants selfed, and among those of 15-chromosome plants pollinated by 14-.

Gates ('09*b*) found all of the 21 chromosomes of the triploid forms which he studied were distributed to the two poles of the heterotypic spindle in groups of 10 and 11, ordinarily, and 9 and 12, occasionally, No evidences of degeneration were recorded. Geerts ('11), on the other hand, found that only 14 of the 21 chromosomes of the triploid hybrids which he examined were regularly distributed in groups of 7 each, the remaining 7 fragmenting and degenerating. The observations of these two workers being so unlike, the following statement was made by Lutz ('12, pp. 404–405): "The evidence does not indicate that we shall find one type of reduction exclusively in 21-chromosome mutant *A*, for example, and another in a sister mutant *B*. It is possible . . . that the type of reduction present in the male and female germ cells of a flower depends upon its position on the plant. . . . For instance, the reduction division in both the male and female germ cells of the first flowers of a triploid plant might be represented by the Gates type almost exclusively, while that of the late flowers on the same branch (or stem) might exhibit chiefly the Geerts type of reduction, or vice versa. Perhaps also, the first flowers of a weak lateral or sub-lateral branch may differ from the first flowers of the stem or a strong basal branch." An interview with Dr. Geerts later revealed the fact that his fixations had been prepared in September and October and that they had been taken from seed-plants, therefore from individuals which had produced their first flowers much earlier in the season; hence it was stated (p. 405) that "This indicates that Geerts's type of reduction appears in the later flowers, and Gates's probably in the earlier ones." Gates ('15*a*, p. 188) has since supported this assumption by the statement that the material from which his studies were made had been collected at the height of the flowering season.

Gates and Miss Thomas ('14) have shown that one of the extra chromosomes of *O. lata* and various *lata*-like forms sometimes degener-

forms, that 8-chromosome male gametes are not produced by the latter. Perhaps all of the 8-chromosome pollen grains of *O. lata rubricalyx* and certain other 15-chromosome forms, whether seemingly good or not, are incapable of functioning.

ate. While they did not tell us whether this occurs more frequently in terminal than in basal buds, it is probable, judging from the evidence produced by the 21-chromosome hybrids mentioned, that the extra chromosomes of 14+-chromosome forms degenerate more frequently in the buds produced near the end of the stem or branch than in earlier ones; or more frequently in the buds of a short, weak lateral or sub-lateral produced near the close of the flowering period of the plant, than in the buds of a vigorous branch.

Since 15-chromosome forms commonly produce no pollen or very little seemingly good pollen, one can exercise but little choice in the selection of pollen-flowers; yet if it were possible to self one of the early flowers of the stem or a vigorous branch (not necessarily one of the first), he might secure a higher percentage of 15-chromosome offspring than is common, and even some 16-chromosome forms. Terminal flowers are avoided for obvious reasons; they are commonly regarded as less vigorous than earlier ones, and those of annual plants are usually produced too late to ripen seeds; furthermore, even those of 14-chromosome forms frequently produce less pollen than earlier ones and such pollen as is produced often contains a low percentage of seemingly good grains. Terminal flowers of 15-chromosome forms or other (14+–28)-chromosome individuals producing but little pollen are usually entirely male-sterile; yet by covering the stigmas of early and late flowers of *O. lata* with *Lamarckiana* pollen and employing some method which will secure the germination of all viable seeds, one should be able to ascertain whether the early flowers produce more 8-chromosome eggs, capable of functioning, than the late.[43]

[43] In "New dimorphic mutants of the Oenotheras" referred to in note 36, de Vries has shown conclusively since the above was written that in the case of the individual *O. cana* which he employed, at least, as high a percentage of offspring duplicating the characters of the mutant parent was produced by seeds derived from selfed terminal buds of the stem and side branch as from those of selfed basal buds of the same parts. If *O. cana* has 15 chromosomes, as is probable, he has shown that the relative number of 8-chromosome female gametes was not less in the terminal, than in the basal, buds of the stem and a side branch of the plant employed. We may find this to be true of all 15–, or even of 14+-chromorome forms in general, but we must not overlook the fact that the germ cells of *O. cana* have not been studied as yet, and it may be that degeneration occurs less frequently in certain 14+-chromosome types, or individuals of a given type, than in others. It may be, furthermore, that chromosome degeneration is less likely to occur in plants having 15 than in those having 21 or 22 chromosomes. De Vries having found that a higher percentage of the offspring of selfed biennial, than of selfed annual, *O. cana* and *O.*

Bartlett ('15*b*, p. 141) says: "Recent discoveries are making it very clear that mutative changes in the chromosome number occur frequently, and that such changes are always associated with a modification in the morphological characters of the plant. In other words, certain mutations are probably dependent upon, or, at any rate, closely associated with, visible changes in the nuclear mechanism. We have every reason to believe, therefore, that the different chromosome numbers of different species were acquired simultaneously with the acquisition of other specific characters."

Gates ('15*b*), as earlier quoted, states that whenever a germ-cell having 8 chromosomes fertilizes a normal germ-cell (and he would doubtless concede the reverse as well), a new form is produced and that one of the most important factors determining the nature of the characters of the new form is probably the peculiar combination of chromosomes received.

It is now quite certain that whenever an offspring is derived from a 14-chromosome form by means of the union of an 8- with a regular or irregular 7-chromosome cell, the offspring will invariably differ from the parent in somatic character as well as in somatic chromosome number. Likewise, it has been shown that whenever an offspring is derived from a 15-chromosome plant by means of the union of two 7-chromosome cells, it will invariably differ from the parent in somatic character as well as in somatic chromosome number. We have seen, however, that unsurmountable difficulties are soon encountered when one attempts to explain mutation on a strictly chromosomal basis.

We may now return to the question of the probable number of chromosomes which were present in *O. semilata* de Vries.

Having found that *O. semilata* de Vries and *O. semilata* Gates are distinct types, Gates ('15*a*, p. 111) concludes regarding the former that "since it bred true it probably had 16 chromosomes," and adds that it will therefore be understood that de Vries's form "is another mutant which probably had 16 chromosomes."

Whether or not *O. semilata* de Vries had 16 chromosomes is in itself a matter of small importance, since the strain has died out and this particular mutant type may never reappear; but the questions which Gates's statements raise are of considerable interest.

scintillans reproduce the characters of the mutant parent, indicates, in the opinion of the writer, that *the extra female chromosome degenerates less frequently in strong biennials than in the less vigorous aunuals and that 8+7 unions occur more frequently than 7+7, when biennials are selfed, for the simple reason that relatively fewer female 7-chromosome gametes are produced.*

It may be asked, Why should we assume that *O. semilata* de Vries had 16 chromosomes rather than 14, or even 15?

We know that most 14-chromosome forms breed true and there is evidence to indicate that a 15-chromosome form may breed true. The *semilata* de Vries whose constancy de Vries tested, appeared among the offspring of *O. lata* (15) × *O. Lamarckiana* (14).[44] As is well known, this cross produces among others, 14-chromosome *O. nanella* and *O. Lamarckiana*, 15-chromosome *O. albida* and 14- or 15-chromosome *O. oblonga*, all of which are said to breed true. The three 16-chromosome mutants described in this communication are the only 16-chromosome forms which have been reported thus far and these three plants exposed no pollen whatever, hence no offspring have been obtained from them and we have no evidence to indicate that 16-chromosome forms are more likely than 15- to breed true. *While it is probable we shall find that most forms having an odd number of chromosomes are inconstant, whether the number be 15 or 15+, it does not follow that forms having an even number of chromosomes may be expected to breed true when the number is in excess of 14, except, perhaps, when this number is twice 14.* It is probable that a *Lamarckiana* offspring or descendant having a double set of the original, parental 14 chromosomes, as is possible in the case of *O. gigas* de Vries, might be more stable than a 16-chromosome offspring, yet it is quite certain that the 28-chromosome mutant offspring of 14-chromosome forms with which we are familiar do not breed true in the same sense as *O. Lamarckiana*. The progeny of *O. gigas* de Vries do not all duplicate the parental individual in every character, as in the case of *O. Lamarckiana*. As is well known, a number of types are found among the offspring, yet since they have many characters in common and since one type, when selfed, seems to produce the same lot of offspring as any other, we speak of this form as constant. Gates's 28-chromosome Palermo strain of *O. gigas* also "showed a considerable range of variation, though not so great as in the Amsterdam race" (p. 121). Heribert-Nilsson's race (Heribert-Nilsson, '12), representatives of which Gates ('15*a*, p. 124) has recently shown to have 28 chromosomes, also produces a variable lot of offspring. Furthermore, Bartlett ('15*c*) has announced that the 28-chromosome mutant offspring of *O. pratincola* which appeared in his cultures, namely *O. pratincola* mut. *gigas* gave a diverse progeny of dwarfs, not a single individual of which resembled the mother. While it appears that we are entitled to regard this form as inconstant, Professor Bartlett, in discussing this question by letter says, "The fact must not be overlooked, however, that this particular individual of *Oe. pratincola* mut. *gigas* belonged to a mass mutating strain."

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[44] De Vries recognized three *semilata* mutants in his cultures. All had *lata* mothers.

Gates ('15*a*, pp. 189–190) described the meiotic distributions of the chromosomes of a 22-chromosome offspring of *O. gigas* × *O. lata rubricalyx*, presumably resulting from a ♀ 14 + ♂ 8 union. Here he found that "the arrangement in the heterotypic telophase is distinctly not into two equal groups of 11 each, but usually (and apparently with much regularity) into 10 and 12." In addition to these, 9-, 11-, and 13-chromosome groups were observed and certain other irregularities of distribution, but these were apparently uncommon. "Hence," he says, "we conclude that a considerable number of the pollen grains will contain only nine chromosomes, although the majority will probably contain 10, 11, or 12."

The behavior of *O. lata* has shown us that although male reduction in a plant may form daughter groups containing different numbers of chromosomes, it does not necessarily follow that more than one type of pollen grain, capable of functioning, will be produced. Thus, Gates and Miss Thomas ('14) have shown that male reduction in *O. lata* usually results in 7–8 distributions of chromosomes, yet there is much to indicate, as we have seen, that only 7-chromosome pollen grains, *capable of functioning*, are produced by this form. Hence the appearance of 9-, 10-, 11- and 12-chromosome groups at various stages of the male reduction process in the 22-chromosome hybrid does not assure us that more than one type, or that any type of pollen grain, capable of functioning, was produced by this form.[45] Gates ('15*a*, p. 213) studied a sample of pollen from this hybrid containing 281 grains and found that 11.4 percent of the grains were "good." It is quite possible that all but one type of grain were eliminated and that only one type of female gamete, capable of functioning in union with the male gamete, was produced. If the fertilization combination (such as ♀ 10 + ♂ 12, or vice versa) resulted in 22-chromosome seeds capable of germinating, or if, regardless of the chromosomal contents of the gametes of both sexes, of the chromosomal combinations which resulted from fertilization, only 22-chromosome seeds were capable of germinating, it is clear that a 22-chromosome hybrid might breed true. On the other hand, if functional male gametes of two or more types (such as 10- and 12-chromosome cells) and a female gamete of a single type (say 10-chromosome cells), or vice versa, were produced, and if a single type of cell of one sex were capable of uniting with two or more types of cells of the opposite sex and of producing seeds capable of germinating, it is quite clear that the 22-chromosome hybrid would not breed true.

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[45] The 22-chromosome offspring of *O. lata* × *O. gigas* sometimes produce small quantities of pollen containing about the same percentage of seemingly good grains as the pollen from Gates's hybrid. I have repeatedly attempted to self these plants, but in every instance have failed to secure a single seed. These results may have been brought about by incompatibility of fertilization combinations, or it may be that the "seemingly good" grains were just as incapable of functioning as the shriveled and distorted ones.

If enough good pollen were produced by 16-chromosome mutant offspring of selfed *O. lata*, *O. Lamarckiana*, or of *O. lata* × *O. Lamarckiana*, to self them, one might expect them to prove less stable than 14-chromosome forms resulting from 7 + 7 unions, since they would contain two extra chromosomes, whether derived from 8 + 8, or 9 + 7 unions. It is quite possible, of course, that a germ-cell combination would be formed which would enable the mutant to breed true, such as ♀ and ♂ 8, ♀ 7 and ♂ 9 or ♀ 9 and ♂ 7, but no evidence has been produced to assure us that such would occur. It would not be at all surprising to find that 16-chromosome forms derived from 8 + 8 unions produce only 8-, or 7- and 9-chromosome female gametes, and, in case viable pollen is ever formed, only 7- male; and that those derived from 9 + 7 unions produce 7- and 9- female, and only 7- male gametes.

Returning to the case of *O. semilata* de Vries, de Vries states ('09, Vol. I, p. 359) that he selfed one of these mutants and obtained 276 offspring. Of this number, 3 were *O. nanella* and 4 *O. lata*. "The remaining plants clearly exhibited the characters of *semilata* and justify the establishment of this form as a constant species." He also pollinated *O. lata* with this *semilata* plant and obtained 105 seedlings, 39 of which were *O. lata*, 1 *O. albida*, 61 *O. Lamarckiana*, 2 *O. nanella* and 2 *O. oblonga* (the first two types having 15, the second two 14, and the fifth, 14 or 15 chromosomes). "These forms," he adds, "and the proportions in which they occur are the same as those which *O. lata* produces when crossed with other species" (meaning, probably, when *O. lata* is pollinated with 14-chromosome species, since sufficient pollen for fertilization purposes is rarely obtained from 15-chromosome forms; however, this statement does not necessarily exclude the latter, since *no evidence has been produced to show positively that 7+-chromosome male gametes, capable of functioning, are produced by any 15-chromosome form*). The results are practically the same as those derived from 15-chromosome *O. lata* × 14-chromosome *O. Lamarckiana*. This certainly indicates that the buds of *O. semilata* de Vries which were employed in this cross, produced only 7-chromosome male gametes capable of uniting with the 7- and 8-chromosome eggs of *O. lata* and producing viable seeds. If this mutant had 16 chromosomes and if only 7-chromosome male gametes were produced by all buds, it is obvious that when this form was selfed, the only eggs which united with the 7-chromosome male gametes and produced seeds capable of germinating in the time which de Vries allowed them, had 9 chromosomes.

All facts and possibilities considered, it seems quite as probable that *O. semilata* de Vries had 14, as 16, chromosomes. The production of pollen by this form and the evidence of constancy, when selfed, do not preclude the possibility of its having had 15 chromosomes.

We may briefly outline our conclusions regarding the factors which determine the constancy or inconstancy of a plant as follows:

It has been shown that somatic chromosome number in Oenothera is constant; therefore, unless 15-chromosome offspring are produced apogamously or unless the chromosomes in excess of 15 are eliminated after fertilization takes place, it is evident that a 15-chromosome form can breed true, *i. e.*, produce offspring having the somatic characters of the parent in every case, only when two gametes having dissimilar chromosome numbers, one odd and the other even, unite and produce viable seed. While not all offspring resulting from such combinations reproduce the parental characters, it is certain that, with the possible exceptions noted, the parental type can be duplicated in no other way. However, as we have seen, *the constancy or inconstancy of a plant is not determined solely by the presence of an even number of chromosomes in the first case, and of an odd, in the second. All depends upon the types of male and female germ-cells produced and the fertilization combinations which result in the production of seeds capable of germinating*. Thus, mutant *A*, having 15 chromosomes, may produce only 8-chromosome gametes, type *a*, of one sex, and only 7-chromosome gametes, type *b*, of the other sex, or, although others are formed by either or both sexes, these may be the only two that are capable of uniting and producing viable seeds. If the 8 + 7 combinations unite gametes which, together, reproduce the parental characters, the plant will, of course, breed true. If they unite other types the plant will prove inconstant, notwithstanding the fact that the offspring, like the parent, will have 15 chromosomes.

C. 16-CHROMOSOME MUTANTS[46]

1. Lata-*like Forms*

The first 16-chromosome mutant recognized at Cold Spring Harbor or elsewhere, was found in a 1908 culture of *O. Lamarckiana* × *O. Lamarckiana*, and the second in a 1910 culture of the same form. Since the somatic chromosome number of the 1908 mutant was ascertained in the winter of 1908–1909 and that of the 1910 plant in the spring of 1911, they were not known to be mutants of particular interest at the time of their growth and were not photographed.

While the two were in no sense identical forms, both have been properly characterized as *lata*-like plants. In common with *O. lata* Nos. 5343 (1908) and 3474 (1910) had crinkled leaves, yellow-green foliage, irregularly shaped buds, and were male-sterile. The leaves of No. 5343, in all stages of development, were conspicuous because of their relatively short and broad leaf-blades and long petioles, but the leaves of No. 3474 were very much narrower and more sharply pointed than those of *O. Lamarckiana lata.* In both cases these differences were very conspicuous in the full-grown rosettes. The true *lata* mutant produced by *Lamarckiana* is usually much shorter than *Lamarckiana*, but No. 3474 was almost as tall as the parental form when full grown, its height being correlated, undoubtedly, with the great distance between nodes—one of the conspicuous characters of the plant. In proportion to the length of the stem, the branches of *O. lata* are rather long, but those of the two mutants were relatively short. *Lamarckiana* and *lata* are almost invariably annual in this climate (when seeds are sown in January and rosettes transplanted in May) and flower quite early, the latter frequently earlier than the former. No. 3474 bloomed quite late, about the middle of August. The buds, seed-capsules, stem and branches of this plant were covered with long hair. The petals of the open flower did not have the ordinary crumpled appearance characteristic of *O. lata*, but were creased longitudinally, much as if the flower had been drawn through a very small finger ring. Many flowers had five or more petals. The stigmas were very irregularly branched, much more so than in *O. lata*, and an anther occasionally bore a rudimentary petal. Somatic metaphase groups from Nos. 3474 and 5414 are shown in Figs. 8*a*, 8*b* and 8*c*.

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[46] The discovery of 16-chromosome mutants in Oenothera was announced with the following statement in 1912 (Lutz, "Triploid mutants in Oenothera," p. 433): "I may anticipate a future report sufficiently to state that I have found many quite distinct types of mutants with 15 chromosomes, and some even with 16." No further information concerning these mutants was given out at that time and the plants were not mentioned again by the writer until referred to in a paper read before the Botanical Society of America in December, 1915, and in the note which followed (Lutz, '16*a*). Gates stated in 1915 ("The mutation factor in evolution," p. 167) that 16-chromosome forms had been described, but since there appear to be no recorded descriptions of such forms antedating the note just mentioned and the paper in hand, it is probable that he referred to the mutants reported in 1912.

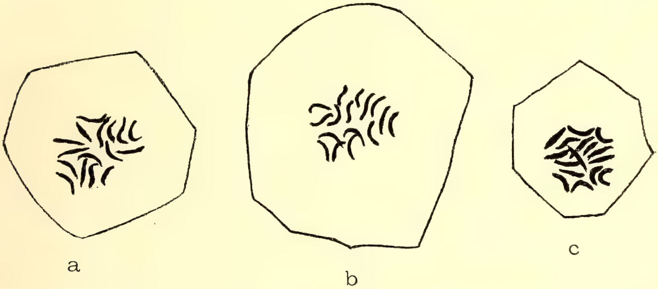

FIG. 8. *a* and *b*, unidentified *lata*-like mutant, plant No. 3474, C.S.H., 1908. Offspring of *O. Lamarckiana* × *O. Lamarckiana*. Polar view of metaphase figures from transverse sections of root-tips, showing 16 chromosomes. *c*, unidentified mutant, plant No. 5414, C.S.H., 1910. Offspring of *O. lata*, selfed. Polar view of metaphase figure from transverse section of root-tip, showing 16 chromosomes.

2. *The Dwarf Form Produced by* O. lata, *Selfed*

This plant, No. 5414 (1910), was abnormal in appearance in all stages of development. It is shown as a greenhouse rosette in Fig. 9*a*, and as a full-grown garden rosette in *b*, the diameter of the latter not exceeding one fourth of the diameter of a full-grown *lata* rosette. The plant came to flower late in the season on a very short stem and it is impossible to state whether this was due to the character of dwarfness (suggested in the rosette) or to a depauperate, abnormal condition.

No. 5414 produced no pollen whatever. A somatic metaphase group from this plant (Figs. 9*a*, 9*b*) is shown in Fig. 8*c*.

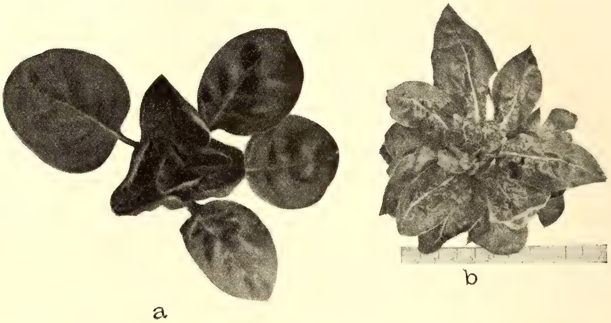

FIG. 9. *a*, plant No. 5414 (see 8 *c*) in greenhouse rosette stage. *b*, same plant in late garden rosette stage. About 1.5 dm. in diameter; growth completed. Note its abnormal appearance.

3. *Origin of the 16-chromosome Condition in Offspring of 14- and 15-chromosome Forms.*

As has been pointed out on preceding pages of this report, the 16-chromosome condition in the three mutants may have arisen through 9 + 7 or 8 + 8 unions; it is difficult to state which is the more probable in either case. If the plant produced by *O. lata* was the product of the first combination, it is probable that it resulted from a ♀ 9 + ♂ 7, rather than a ♀ 7 + ♂ 9, union.

The 1908 and 1910 *Lamarckiana* mutants were far more suggestive of *O. lata* than the 1910 offspring of selfed *lata*. The latter could not be designated as a *lata*-like plant. It might be suggested that the two former may each have arisen from the union of two 8-chromosome gametes bearing *lata* characters and the latter from a ♀ 9 + ♂ 7 union, but we should then be required to explain why the *Lamarckiana* mutants did not have duplicate vegetative characters.

The 1908 plant was grown during the first season in which the writer studied the vegetative characters of various plants of the *Lamarckiana* group with particular care, hence minor differences between No. 5343 and ordinary *lata* may have been overlooked. The records refer merely to the distinguishing characters of the leaf, branching habits, etc. If this mutant bore signs of abnormality or irregularity such as were observed in the other two forms, the fact was not observed; yet the indications of abnormality in Nos. 3474 and 5414 lead us to conclude that the gametic combination or combinations which produced these two plants may have been less "compatible" than the combination which produces *O. lata*.

SUMMARY

1. The primary object of this series of three reports, of which the one in hand is the second, is to discuss, in the light of the Cold Spring Harbor and Louvain studies of Oenothera, certain theories and conclusions which Gates has advanced from time to time and which Gates and Miss Thomas have based upon the results of their investigations.

2. *O. Lamarckiana* mut. *lata*, long believed to have 14 chromosomes, is now known to have, invariably, 15. The researches of Gates and Miss Thomas appear to have led them to conclude, further, that the presence of the extra chromosome in the somatic cells of 15-chromosome offspring of 14-chromosome forms is invariably associated with the presence of *lata* or *lata*-like vegetative characters. Later, Gates recognized the fact that his 15-chromosome mutant *O. incurvata* is quite different from *O. lata*, as is also a 15-chromosome form which de Vries reported. That he is loth to concede that these discoveries render untenable certain earlier statements of Gates and Miss Thomas is indicated by the statement that "It is perhaps not inappropriate to speak of all these mutants as belonging to the *lata* series, or series with an extra chromosome." The primary object of this paper, therefore, is not only to emphasize the fact that these two mutants cannot be regarded as *lata*-like forms, but to show that many other 15-chromosome mutant offspring of 14- and 15-chromosome forms, are not *lata*-like.

3. The distinct types of mutants which the Cold Spring Harbor and Louvain studies have shown to have 15 chromosomes, are: (1) *O. lata*, (2) *O. albida*, (3) *O. bipartita*, (4) type 5509 (supposed to be modified *O. oblonga*)—all *Lamarckiana* mutants. (5) *O. nanella lata*, produced by *O. Lamarckiana*, *O. nanella*, *O. lata* × *O. Lamarckiana*, etc. (6) *O. subovata*, found in cultures of *O. Lamarckiana* and *O. lata* × *O. Lamarckiana*. (7) A dwarf type, 2256, produced by *O. nanella*, selfed. (8) Type 4499, found in cultures of *O. lata* × *O. Lamarckiana* and *O. lata*, selfed. (9) *O. exilis*, (10) *O. exundans* and (11) type

5365, all found in cultures of *O. lata*, selfed. In addition to the foregoing, type 2806, having many points in common with type 5509, also has 15 chromosomes.

4. Of the above 12 types (11 of which were quite distinct) now known to have 15 chromosomes, only two are *lata*-like; namely, *O. lata* and *O. nanella lata*.

5. Certain somatic characters of many mutant offspring of *O. Lamarckiana* × *O. Lamarckiana* and *O. Lamarckiana*, selfed, indicate that a very large percentage of the mutant offspring of *O. Lamarckiana* have 15 chromosomes and that a larger number of 15- than of 14-chromosome mutant offspring are produced by this form. Not only does there appear to be a larger number of distinct types of 15- than of 14-chromosome mutants, but a higher percentage of 15- than of 14-chromosome individuals produced by *O. Lamarckiana*. Only a small percentage of the former may be classed as *lata*-like, or as *semilata*-like, forms.

6. While *lata*-like forms are commonly characterized by 15 chromosomes, three distinct types have been found in Cold Spring Harbor cultures with 16 chromosomes. Two appeared in separate cultures of *O. Lamarckiana* × *O. Lamarckiana* (1908 and 1910) and one among the offspring of *O. lata*, selfed (1910).

7. Owing to the fact that 15-chromosome forms are very often male-sterile, or produce but little pollen capable of functioning, their constancy has not been tested upon an extensive scale. 15-chromosome mutants *O. lata*, *O. semilata* Gates, *O. lata rubricalyx*, *O. bipartita* and 15(?)-chromosome *O. elliptica*, are known to be inconstant, while de Vries's researches indicate that 15-chromosome *O. albida* and 14- or 15-chromosome *O. oblonga* are constant.

8. The evidence available at present indicates that most 14-chromosome forms are constant and most 15-chromosome forms inconstant. Furthermore, the available evidence indicates that inconstancy is commonly associated with the 14+-chromosome condition. It seems, however, that forms having twice 14 chromosomes are more likely to be constant in the same sense that de Vries's *O. gigas* is constant, than those having more than 14, but fewer than 28, chromosomes.

9. While it is probable that we shall find that most forms having an odd number of chromosomes are inconstant, whether the number be 15 or 15+, it does not follow that forms having an even number

of chromosomes may be expected to breed true when the number is in excess of 14, except, perhaps, when this number is twice 14. Hence the fact that *O. semilata* de Vries bred true, scarcely warrants the conclusion that this form probably had 16 chromosomes. We now know that 14-chromosome forms usually breed true and the evidence indicates that an occasional 15-chromosome form is also perfectly constant, while there are no records to show that offspring have been obtained as yet from forms known to have 16 chromosomes. It therefore seems quite as probable that *semilata* de Vries had 14, or even 15, as 16, chromosomes.

10. Since somatic chromosome number has been shown to be constant in Oenothera, it is clear that unless 15-chromosome offspring are produced apogamously, or unless the chromosomes in excess of 15 are eliminated after fertilization takes place, 15-chromosome forms can breed true, *i. e.*, produce offspring having the somatic characters of the parent in every case, only when two gametes having dissimilar chromosome numbers, one odd and the other even, unite and produce viable seeds. While not all offspring resulting from such combinations reproduce the parental characters, it is certain that, with the exceptions noted, they can be duplicated in no other way.

11. The constancy or inconstancy of a plant is not determined solely by the presence of an even number of chromosomes in the first case and of an odd in the second. All depends upon the types of male and female germ-cells produced and the fertilization combinations which result in the production of seeds capable of germinating.

12. As a rule, larger quantities of seeds are obtained from 14-chromosome forms selfed, or pollinated by other 14-chromosome forms of the same, or different species, than from 14+-chromosome forms selfed, or pollinated by other 14+-chromosome plants of the same, or different species, particularly if the 14+-chromosome individuals have more than 14, but fewer than 28, chromosomes; furthermore, higher percentages of germination are usually secured from the former than from the latter when seeds not more than one year old are sown in pans of sterilized soil in January and are kept under ordinary greenhouse conditions.

13. The number of seeds produced by a form and the ability of the seeds to germinate, at least within the time limits specified, are factors which appear to be associated with the chromosome number of the plant, or numbers of the plant, producing them.

14. The ability of a seed to germinate seems to depend, not wholly, but to a certain extent, upon the number of chromosomes which it bears, and, possibly, in accordance with Gates's suggestion, upon the compatibility or incompatibility of the chromosomal combination which the number represents.

15. The ability of a seed to germinate appears to be directly associated with its own chromosome number and only indirectly with that of its parent, for the results derived from the Cold Spring Harbor and Louvain studies indicate that 14-chromosome seeds of 14+-chromosome forms germinate quite as readily as 14-chromosome seeds of 14-chromosome forms.

16. Plants having more than 14, but fewer than 28, chromosomes are more inclined to be male- than female-sterile. Just why this is so, is not yet clear.

LAFAYETTE, INDIANA

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THE INFLUENCE OF TEMPERATURE ON THE GROWTH OF ENDOTHIA PARASITICA

Neil E. Stevens

In an earlier paper (4) the writer discussed the influence of certain climatic factors on rate of vegetative growth and production of ascospores in *Endothia parasitica* (Mur.) And. and And. From the data then available it was concluded that the rate of lateral growth of cankers on *Castanea dentata* (Marsh) Borkh. was directly dependent on the amount and duration of temperatures favorable for growth and apparently unaffected by the amount or frequency of rainfall. Ascospore production on the other hand seemed to be dependent chiefly on the presence of abundant moisture. The data on which these conclusions were based were obtained from observations made at a series of stations extending from Concord, N. H., to Charlottesville, Va., during the summers of 1914 and 1915.

Although it has been necessary to abandon several of the stations because of the increasing abundance of the chestnut blight, observations have been continued in six localities. The results seem to warrant a brief statement. As the methods employed have been fully discussed in the earlier paper they need not be considered here.

Rate of Lateral Growth

The abundant rainfall of the summer of 1915 resulted in the production of ascospores on practically all the inoculations at every station, consequently no further data on this point could be obtained.

Table I

Lateral Growth of Cankers of Endothia parasitica in Various Localities

Locality	Elevation (in Feet)	Year Ending 1916	Centimeters
Concord, N. H.	350	May 18	14
Williamstown, Mass	711 (900)	May 22	15
Amherst, Mass. (two stations)	222	May 17	17
Woodstock, N. Y.	1,000	May 24	15
Washington, D. C.	112 (400)	May 4	21
Charlottesville, Va.	854	April 8	23

The lateral growth of the cankers at the various stations is given in Table I. The amount given is, as in the earlier paper, an average of all the normal appearing cankers from ten inoculations.

TABLE II

Total Precipitation (in Inches)

	Concord, N. H.	Williamstown, Mass.	Amherst, Mass.	Mohonk Lake, N. Y.	Washington, D. C.	Charlottesville, Va.
April, 1915	—	—	—	—	—	0.49
May, 1915	0.99	1.46	1.20	2.54	2.18	2.44
June, 1915	1.39	1.73	3.00	2.65	6.58	5.32
July, 1915	10.29	9.37	9.13	8.24	3.21	3.71
August, 1915	6.26	4.47	8.28	7.94	7.00	7.83
September, 1915	1.21	3.44	1.37	2.87	1.39	2.38
October, 1915	3.02	2.71	2.89	2.50	3.72	4.39
November, 1915	2.97	2.03	2.20	1.22	0.93	1.92
December, 1915	3.41	5.03	5.86	8.90	2.80	3.54
January, 1916	1.22	2.05	2.56	2.64[1]	1.57	1.34
February, 1916	4.18	1.53	5.27	5.54	2.87	4.10
March, 1916	3.01	3.51	3.97	5.76	2.80	4.23
April, 1916	2.96	2.48	3.69	4.05	2.96	2.35
May, 1916	3.95	3.52	3.21	2.93	2.30	
Total for year ending	5-31-16 43.83	5-31-16 41.87	5-31-16 51.43	5-31-16 55.24	5-31-16 38.13	3-31-16 41.69

TABLE III

Number of Days with Precipitation .01 Inch or More

	Concord, N. H.	Williamstown, Mass.	Amherst, Mass.	Mohonk Lake, N. Y.	Washington, D. C.	Charlottesville, Va.
April, 1915	—	—	—	—	—	4
May, 1915	9	8	11	9	11	10
June, 1915	10	11	8	7	14	10
July, 1915	16	20	14	18	13	12
August, 1915	15	15	14	10	18	15
September, 1915	5	6	7	6	7	7
October, 1915	9	10	7	4	13	14
November, 1915	16	13	7	3	8	4
December, 1915	12	16	12	6	9	6
January, 1916	8	12	9[2]	9	13	10
February, 1916	13	14	14	6	10	9
March, 1916	10	13	12	8	10	12
April, 1916	15	13	13	17	13	9
May, 1916	11	10	12	11	11	
Total for year ending	5-31-16 140	5-31-16 153	5-31-16 129	5-31-16 105	5-31-16 139	3-31-16 113

[1] Data taken from West Point. Report from Mohonk Lake missing.

[2] Data taken from West Point. Report from Mohonk Lake missing.

Comparison of the amount of growth at the various stations for the year ending in the spring of 1916 with that in the same localities for the years ending in May and in August, 1915, shows a general agreement, although the growth at Charlottesville was only 23 centimeters for the year ending in April, 1916, as against 25 centimeters for the year ending in April, 1915.

Relation of Rainfall to Growth

In considering the influence of rainfall on vegetation both the total amount of precipitation and its frequency must be taken into account. Tables II and III give the monthly totals and number of days with over .01 inch of rain for each month during the period under consideration, together with the totals of the twelve calendar months most nearly coinciding with the period for which growth was actually measured. From these it is apparent that no causal relation exists between the amount or the frequency of rainfall and the rate of growth. For example, the total rainfall for the year was very nearly the same at Williamstown as at Charlottesville but the growth was fifty percent greater at the latter point. Even more significant is the fact that although the rainfall at Concord, Williamstown, and Mohonk Lake was much greater for the year ending in May, 1916, than for the year ending in May, 1915, a difference of about twenty inches at Mohonk Lake, there was no perceptible difference in the rate of lateral growth. A comparison of the number of days with rain and of the rainfall for the warmer months at the various stations also fails to show any relation between rainfall and rate of growth.

Methods of Computing Temperature Efficiency

No method of interpreting climatological temperature data with reference to the influence of temperature on plant growth has yet been devised. The monthly and annual mean temperatures given in the climatological reports are obviously of little use for this purpose. Length of frostless season is of course important for many plants but has little or no significance for a fungus like *Endothia parasitica*, whose growth is by no means confined to the frostless season. In order that the temperature data given in meteorological reports may be really useful in plant climatology, it is necessary to obtain some kind of temperature indices which will express the effect of temperatures on plant growth.

Such temperature indices must take into consideration both the daily temperature means and the frequency with which those means occur during the period under consideration.

Among the methods suggested for attaining this desired end the one most widely used has recently been designated by Livingston (3) as a summation of remainder indices. This method consists in subtracting a certain assumed minimum from each daily mean temperature and summing the remainders. A second[3] method was suggested a few years ago by the Livingstons (2). It is based on the supposition that plant growth follows the chemical principle of van't Hoff and Arrhenius, which states that the velocity of many chemical reactions approximately doubles with each increase in temperature of 10° C. On this basis these authors have computed efficiency indices for the various temperatures, using 40° F. as unity.

The two methods just described are open to the theoretical objection that they fail to take into account the fact that the highest temperatures experienced in nature do not permit as rapid growth as somewhat lower temperatures.

In an attempt to overcome this defect Livingston (3) has recently published a series of temperature efficiency indices based on actual physiological experiment. Using the data obtained by Lehenbauer (1) for the average hourly rates of elongation of shoots of seedling maize plants when exposed for periods of twelve hours to temperatures of 12 to 43° C., he has derived a series of indices which express the average hourly growth rate for each degree C. or F. in terms of the growth rate for 4.5° C. (38° F.) considered as unity.

This series differs from the two described above in that the indices gradually increase up to a certain point (89° F.) and then decrease at higher temperatures. The optimum temperature thus indicated is of course that of the maize seedling under the conditions of Lehenbauer's experiment and is higher than any daily mean reached during this investigation. Moreover, the rate of increase in index value between the minimum and optimum for growth is much more rapid in the physiological series than in either of the other series. So far as the present study is concerned this constitutes the chief difference between this system and the other two.

---

[3] These methods of interpreting temperature data are rather fully discussed by Livingston (2 and 3) and their application to the study of *Endothia parasitica* by the writer (4).

Relation of Temperature to the Growth of *Endothia parasitica*

As in my earlier paper, the temperatures as given by the U. S. Weather Bureau reports for the various localities under consideration were computed according to the methods of summing remainder indices and summing exponential indices. The results are given in Table IV, and Figure 1. The graph expresses the rate of growth of the fungus and the temperatures at the various localities in percentage of that at Concord, growth and temperature at Concord being considered 100 percent. The results of computing temperature by these two methods are closely comparable, the curves being nearly parallel

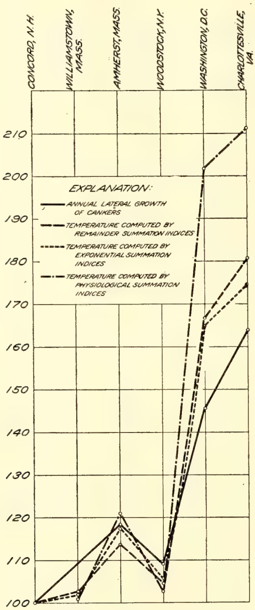

Fig. 1. Lateral growth of cankers of *Endothia parasitica* and temperature computed in various ways for the year ending in May, 1916. All data expressed in percentage of that at Concord.

throughout their length. These two curves are in turn very similar to the curve of growth, although they rise somewhat more rapidly in the more southern localities. This is in general the same relation which was found to hold for the years ending in May and in August, 1915, at a still larger number of localities. Taken together these results furnish a considerable body of evidence that either of these methods of calculation expresses satisfactorily the relation between air temperature and the growth of *Endothia parasitica* within this area.

In computing the physiological temperature efficiency the daily mean temperatures were calculated by the formula mean = ½ (maximum + minimum). For this mean temperature the equivalent index from Livingston's (3) Table II, p. 406, was substituted and the sums of these daily indices considered the index for the year.

Table IV

Lateral Growth of Cankers of Endothia parasitica and Temperatures Computed in Various Ways for the Year Ending in May, 1916

	Growth in Centimeters.	Percent	Remainder Summation Indices	Percent	Exponential Summation Indices	Percent	Physiological Summation Indices	Percent
Concord, N. H.	14	100	2,967	100	366	100	5,514	100
Williamstown, Mass.	15	107.1	3,038	102.4	373	101.9	5,576	101.1
Amherst, Mass.	17	121.4	3,380	113.9	431	117.8	6,673	121.0
Woodstock, N. Y.	15	107.1	3,100	104.6	386	105.5	5,632	102.2
Washington, D. C.	21	150.0	4,976	167.7	603	165	11,080	201.1
Charlottesville, Va.	23	164.3	5,366	180.7	636	174	11,620	211

The results are given in Table IV. It will be observed that the physiological temperature indices rise considerably faster from north southward than do the summation indices, and that accordingly they correspond rather less well with the rate of growth of the fungus.

It is of course not surprising that the results obtained from the use of the physiological temperature indices given by Livingston should not more nearly approximate the growth of *Endothia parasitica,* since, as Livingston correctly points out (p. 407), the indices are based upon tests of only a single plant species, maize, and from the growth of seedlings, and it is entirely probable that they are not even approximately true for plants of some other species. On the other hand, when the necessarily approximate nature of many of the data are considered, the agreement between the curve of growth of cankers of

Endothia parasitica and those showing the temperature of the various localities is remarkable. This together with the evident lack of agreement between the rate of growth and the amount of rainfall, strongly suggests that the rate of growth of this fungus while growing as a parasite on *Castanea dentata*, is influenced chiefly by temperature.

The data presented in this and the preceding paper indicate clearly that the growth of the chestnut-blight fungus is more rapid in the southern portion of its present range than in the region farther north. Unless some unforeseen factor checks its development, the disease may reasonably be expected to spread still more rapidly as it advances southward.

SUMMARY

The lateral growth of cankers of *Endothia parasitica* on *Castanea dentata* in various localities was about the same for the year ending in May, 1916, as for the year ending in May, 1915.

Neither amount nor frequency of rainfall seems to have any influence on rate of lateral growth. Wide differences in the rainfall for the two years produced no change in rate of growth.

The temperature for the period under investigation was computed according to the systems of "remainder summation indices," "exponential summation indices," and "physiological indices." Of these the last seems to agree least well with the rate of growth of *E. parasitica.*

The first two systems give practically identical results.

The agreement between the curves of temperature and of growth is so close as to indicate that temperature is the chief climatic influence in determining the rate of growth of *Endothia parasitica.*

INVESTIGATIONS IN FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

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CONTENTS

Matroclinic inheritance in mutation crosses of Oenothera Reynoldsii. CARL D. LA RUE AND H. H. BARTLETT 119

Duration of leaves in evergreens . . . . VINNIE A. PEASE 145

The relation between evaporation and plant succession in a given area. FRANK C. GATES 161

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MATROCLINIC INHERITANCE IN MUTATION CROSSES OF OENOTHERA REYNOLDSII[1]

CARL D. LA RUE AND H. H. BARTLETT

INTRODUCTION

This paper is concerned primarily with the peculiar type of inheritance exemplified among the mutations of *Oenothera Reynoldsii,* a species elsewhere described as showing the phenomenon of "mutation *en masse,*" or mass mutation. It has been found that the mutations characteristic of mass mutation in this species, when crossed among one another, or with the parent form, give crosses which in general conform exactly to the type of the pistillate parent, quite regardless of which way the cross may have been made.

De Vries[2] has shown that in *Oenothera Lamarckiana,* the most thoroughly studied of the evening-primroses, the total number of mutations lies in the neighborhood of 2.2 percent. Certain mutations from *Oe. Larmackiana* are themselves more mutable than their parent. Thus *Oe. lata* produces twice, and *Oe. scintillans* three times as many mutations as *Oe. Lamarckiana* itself. Before the discovery of mass mutation in *Oe. Reynoldsii* and *Oe. pratincola,* a form was considered highly mutable if its progeny contained as many as five or six percent of mutations. Aside from *Oe. Lamarckiana,* however, only one species, *Oe. biennis,* had been extensively grown for the detection of mutability,

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[1] Prior to 1915 the work upon which this paper is based was carried on by the Office of Physiological and Fermentation Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and since then by the University of Michigan. Published by permission of the Secretary of Agriculture.

Papers from the Department of Botany of the University of Michigan, no. 155.

[2] De Vries, Gruppenweise Artbildung, p. 329 *et seq.*

[The **Journal** for February (**4**: 53–118) was issued Feb. 17, 1917.]

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and this species had been shown by Stomps[3] and De Vries[4] to be less mutable than *Oe. Lamarckiana*. In the recently discovered mass-mutating species the number of mutations may rise to almost 100 percent of the progenies.

The elementary species that have thus far shown mass mutability are both segregates from the collective species that passes in our floras as *Oenothera biennis*. True *Oe. biennis* seems to be found in America, but the records in regard to its occurrence have not yet been published. It is therefore not incorrect to state that the species (in the narrow sense) is definitely known only in Europe, where it occurs as an introduced weed. The name *Oe. biennis* has been applied correctly by De Vries and Stomps, but very loosely indeed by American geneticists, with the result that the literature is considerably confused. *Oe. Reynoldsii* and *Oe. pratincola* are two, among a number of segregates from the collective species of the floras, that have been described and named[5] for the purpose of keeping a clear record of the genetical experiments that are being carried out with them. They are not recognized in current systematic works.

The first paper dealing with *Oenothera Reynoldsii*[6] was written before any mutation crosses had been made. It was therefore only natural to suggest that the whole series of mutations to which it was giving rise were probably Mendelian recessives. Work on the closely related segregate *Oe. pratincola* shortly afterward disclosed the fact that the mutations characteristic of mass mutation were not Mendelian recessives, but showed matroclinic inheritance in crosses with their parent form.[7] It has now been determined that the first suggestion in regard to the mutations of *Oe. Reynoldsii* was entirely erroneous, since they likewise show matroclinic inheritance. Although the special purpose of this paper is to present the data in regard to matroclinic inheritance, there is one other striking discovery which it is possible to announce at this time, namely, that in *Oe. Reynoldsii*, as well as in *Oe. pratincola*, the occurrence of mass mutation is associated with a remarkable increase in seed sterility. This very significant fact is being made the subject of further study. The degree of seed sterility in mass-mutant *Oe. Reynoldsii* is much greater than in *Oe. pratincola*, and is so marked that otherwise indistinguishable individuals, the one stable, the other mass-mutant, can easily be distinguished by an examination of the seeds.

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[3] Stomps, Theo. J., Mutation bei *Oenothera biennis* L., Biol. Centralbl. **32**: 521–535. 1912; Parallele Mutationen bei *Oenothera biennis* L., Ber. Deutsch. Bot. Ges. **32**: 179–188. 1914.

[4] De Vries, H., The Coefficient of Mutation in *Oenothera biennis* L., Bot. Gaz. **59**: 169–196. 1915.

[5] Bartlett, H. H., Twelve Elementary Species of Onagra, Cybele Columbiana **1**: 37–56. 1915.

[6] Bartlett, H. H., Mutation *en masse*, Amer. Nat., **49**: 129–139. 1915.

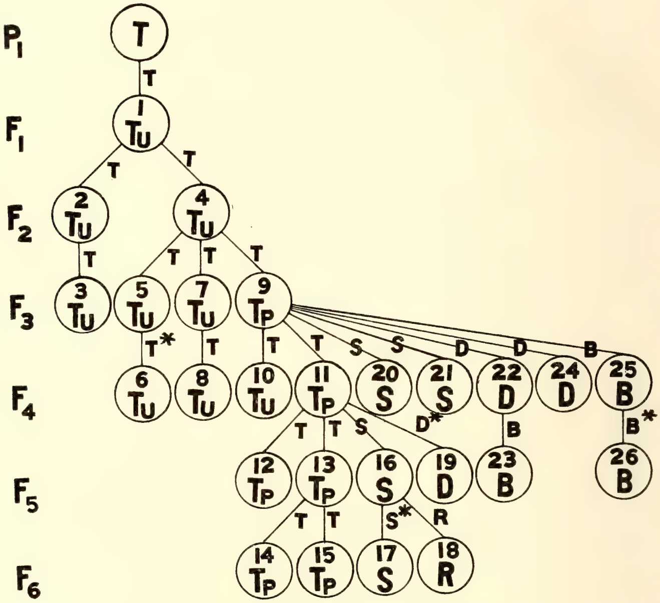
[7] Bartlett, H. H., Mass mutation in *Oenothera pratincola*, Bot. Gaz., **60**: 425–456. 1915.

Without going into detailed repetition of data published in the former paper on *Oe. Reynoldsii*, it may be recalled that the wild form of the species, f. *typica*, has given rise to the derivatives mut. *semialta*, mut. *debilis*, and mut. *bilonga*. The f. *typica* is remarkable in that it exists in two morphologically identical phases, one of which is relatively stable, whereas the other is mass-mutant, giving rise to polymorphic progenies containing all of the mutations enumerated, as well as others which have not yet been carefully examined. Mut. *semialta* was so named because the plants of the early cultures, grown in Maryland, were about half as high as f. *typica*. The cultures of the season of 1916, grown in Michigan under other environmental conditions, did not show so great a disparity in height, but in other respects the forms were no less distinct than before. The shape of f. *typica* is depressed-conical, because of the long, widely spreading lower branches, whereas mut. *semialta* has relatively erect lower branches and is therefore somewhat cylindrical rather than conical in shape. Mut. *debilis* is a weak dwarf with much reduced foliage. Mut. *bilonga* was so named because its fruits are twice as long as those of mut. *semialta*, which it closely resembles in form and stature. In other respects, however, it will be shown that mut. *bilonga* more closely resembles mut. *debilis*, from which it springs, than mut. *semialta*.

All the mutations come true from seed, except that mut. *semialta* is capable of giving rise to mut. *debilis*, and that the latter may in turn give rise to mut. *bilonga*. Mut. *semialta* has once thrown a mutation which will be known as mut. *rigida*. It came entirely true in a large progeny grown in 1916, and will receive a larger share of attention in a future paper. A few other types have appeared in the cultures, but it has not been possible to obtain seeds from them.

Summary of the Cultures

Figure 1 is a chart giving the pedigree of all the cultures of *Oe. Reynoldsii* and its mutations that have thus far been grown from self-pollinated seeds. Each progeny represented in the chart has a key number which serves to identify it with the detailed analysis of the same progeny in Table I. Several of the earlier progenies were not as large as seemed desirable, on which account supplementary cultures were in several cases grown a year or two later from any seeds that had been left over. Such division of progenies between two seasons has provided a very desirable check on the classification of the plants,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16 S
19 D
23 B
26 B
T
T
S*
R
14 Tp
15 Tp
17 S
18 R

FIG. 1. Pedigree of *Oenothera Reynoldsii* and its mutations. Each numbered progeny is represented by a circle. T=*typica*. S=*semialta*. D=*debilis*. B=*bilonga*. R=*rigida*. T_u=uniform culture of *typica*. T_p=polymorphic culture containing *typica*. Letters on the lines leading to circles indicate the parentage of the cultures. A star (*) indicates a plant used as a parent for the crosses referred to in Table II.

TABLE I

Summary of the Pedigree of Oenothera Reynoldsii and Its Mutations, Excluding Crosses, up to and Including the Season of 1916

Key No. (See Fig. 1)	Generation from Foundation of Pedigree	Generation from Appearance of Mutation	Culture No of Parent	Identity of Parent	Seeds Planted	Germinations		Plants Retained to Maturity	*Typica*	*Semi-alta*	*Rigida*	*Debilis*	*Bilonga*	Other Mutations
						No.	%							
1	F_1	..	P	*typica*	..	..	..	10	10	..	..	..	..	..
2	F_2	..	91	*typica*	..	..	..	24	23	..	..	..	..	1
3	F_3	..	91–2	*typica*	..	..	..	110	110	..	..	..	..	..
4	F_2	..	89	*typica*	..	..	..	105	104	..	..	..	..	1
5	F_3	..	89–53	*typica*	500	419	82%	100	100	..	..	..	..	..
6	F_4	..	89–53–1	*typica*	750	538	71%	99	98†	..	..	..	..	1
7	F_3	..	89–85	*typica*	870	733	84%	100	100	..	..	..	..	..
8	F_4	..	89–85–40	*typica*	644	382	59%	100	100	..	..	..	..	..
9	F_3	..	89–3	*typica*	..	..	..	79	29	31	..	18	1	..
10	F_4	..	89–3–4	*typica*	..	..	..	100	100	..	..	..	..	..
11	F_4	..	89–3–13	*typica*	2,500	..	..	24	5	13	..	5	1	..
						111	4.4%	105	20	61	..	21	3	..
12	F_5	..	89–3–13–14	*typica*	1,265	64	5.1%	61	12	35	..	11	..	2
13	F_5	..	89–3–13–15	*typica*	2,000	92	4.6%	90	22	50	..	13	1	4
14	F_6	..	89–3–13–15–50	*typica*	612	14	2.3%	13	9†	2	..	..	..	2
15	F_6	..	89–3–13–15–79	*typica*	472	20	4.3%	20	3†	13	..	3	...	1
16	F_5	F_1	89–3–13–4	*semialta*	658	584	88.7%	50	..	47	1	2	..	..
17	F_6	F_2	89–3–13–4–16	*semialta*	962	597	62%	100	..	100	..	..	..	..
18	F_6	F_1	89–3–13–4–43	*rigida*	1,891	1,360	72%	200	..	..	200	..	..	..
19	F_5	F_1	89–3–13–134	*debilis*	832	62	7.5%	62	..	..	..	60	2	..
20	F_4	F_1	89–3–22	*semialta*	..	..	..	46	..	41	..	5	..	..
21	F_4	F_1	89–3–24	*semialta*	..	..	..	87	..	83	..	4	..	..
22	F_4	F_1	89–3–21	*debilis*	..	..	..	85	..	..	..	84	1	..
23	F_5	F_1	89–3–21–85	*bilonga*	1,084	375*	34.6%*	375	..	..	..	..	375	..
24	F_4	F_1	89–3–25	*debilis*	..	..	..	43	..	..	..	42	1	..
25	F_4	F_1	89–3–53	*bilonga*	815	196*	24%*	190	..	..	..	..	189	1
26	F_5	F_2	89–3–53–22	*bilonga*	750	194*	25.9%*	150	..	..	..	..	150	..

* Germination number and percent include only green plants. See p. 127.

† The *typica* individuals divide into two classes, differing only in habit of growth, either annual or biennial. Both come to maturity in one season, but the biennial individuals stay in the rosette stage longer, and therefore produce more robust plants at time of flowering.

but has brought it about that the different lines composing the cultures have not all been carried the same number of generations since the foundation of the pedigree in 1910. The oldest lines have now been carried through six generations by self-pollination.

At the close of the season of 1914 the cultures of *Oe. Reynoldsii* had been maintained for four generations, and the pedigree, as summarized in the former paper,[6] showed clearly that the individuals of f. *typica*, externally alike, were of two kinds, giving rise, respectively, to uniform and polymorphic progenies. It had been shown, also, that both kinds of f. *typica* occurred in polymorphic progenies. One point, however, was still obscure. The original mass-mutant individual of f. *typica* had been the only plant of its generation self-pollinated for continuing the line, and it was therefore uncertain whether the sister plants of the same culture would have resembled it in giving rise to polymorphic progenies or would have given uniform progenies. In other words, was the original mass-mutant individual of f. *typica* itself of the nature of a rare physiological mutation? In order to answer this question it was necessary to go back to old seeds and retrace two generations. This has been done, with the result that two sister plants of the original mass-mutant individual have given two generations of uniform progeny. (It should be noted that here, as well as in the explanation of the pedigree, Figure 1, a progeny is for convenience termed uniform, as opposed to polymorphic, even if it contains a few mutations, provided the mutability was unaccompanied by unusual seed sterility. The sporadic mutations that have appeared in so-called uniform progenies have in no case been those characteristic of mass mutability. No confusion can possibly arise from this terminology for the reason that Table I gives a detailed analysis of every culture concerned in the experiments.) It is therefore possible to conclude that mutating and non-mutating individuals of f. *typica* may occur together in either uniform or polymorphic progenies. In the former case the mutating individual must itself be regarded as a physiological mutation, or perhaps even as a premutation in the sense of De Vries.[8]

Premutation, according to De Vries, is the process of preparation for mutation. In forms showing ordinary mutability the various mutational types occur in every sufficiently large progeny in every generation, and the process of premutation must therefore be assumed to have taken place far back, and to have brought about a hereditary change by virtue of which all individuals of the line became mutable. The phenomena are not quite comparable in the case of mass-mutant *Oe. Reynoldsii*, and lead one to wonder if the change in the genetic physiology of the original individual of mass-mutant *Oe. Reynoldsii* may not have been a premutation accidentally detected at the actual time of origin. Speculation on such a point, however, will hardly be worth while until the investigations shall have been pushed much further than they have been as yet.

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[8] De Vries, Gruppenweise Artbildung, pp. 9 and 10, 335, 346.

To those who may desire to explain the mutations of *Oe. Reynoldsii* on a Mendelian basis the facts are very refractory. The lines have been grown from guarded seeds since 1911, and have probably been self-pollinated much longer, for the species is one of the smaller-flowered self-pollinating types, producing abundant pollen that is liberated on the receptive stigma a day, or even two days, before the flowers open. If the wild parent plant had been an F_1 hybrid, or a heterozygote of a later generation, the first generation in the garden should have shown segregation, whereas the first polymorphic progeny was obtained two generations later. An explanation based on the multiple factor hypothesis is blocked by the fact that the mutations do not act as Mendelian recessives, but show strict matroclinic inheritance when crossed with the parent type.

SEED STERILITY OF THE MASS-MUTANT INDIVIDUALS

Returning to the problem presented by the two types of individuals of f. *typica*, we see from Table I that there is at least one character by which they may be distinguished. All those plants giving rise to uniform progenies have reasonably good seeds, relatively many of which (58 to 84 percent) readily germinate. Those giving rise to polymorphic progenies, on the contrary, have very poor seeds, few of which (2 to 5 percent) are capable of germinating.

When the seeds for two of the polymorphic progenies (Nos. 11*b* and 12 in Table I) were counted off it was found that only about 5 percent of the seed-like structures were actually perfect seeds with a good embryo. The remainder were either empty shells, or else contained a small amount of yellowish disintegrated tissue. Many were examined. It is therefore certain that the low germinability of the seeds that yield polymorphic cultures is not to be attributed to delayed germination. If a cytological study now in progress throws any light on the reason for the seed sterility, we may be well on the way to an understanding of mass mutation. Certainly there is likely to be some causal relationship between such closely associated phenomena.

One must of course take into consideration the possibility that the defective seeds represent zygotes of f. *typica* that failed to develop. Reckoned from the total number of seed-like structures sown, rather than from the number of plants obtained from them, the proportion of mutations in the polymorphic progenies would not be at all unusual. We are not inclined to believe, however, that any such explanation is the right one. Why should the *typica* zygotes in one case develop into uniformly strong and viable embryos, but in another case, environmental conditions remaining the same, fail to produce even mature embryos? Moreover, if there were no essential difference between uniform and polymorphic progenies other than the failure of *typica* zygotes to develop, why should the mutations found in the polymorphic progenies be characteristic of the latter? It may be urged that the evidence is not sufficiently clear that the non-mass-mutant individuals might not throw mutations *semialta*, *debilis* and *bilonga* if grown in sufficiently large cultures. For the present it must suffice to say that they have not done so, although we are keenly aware of the fact that the cultures have not been as large as one would wish for convincing evidence on this point. Very much larger cultures to test this question are planned for next year. It should be remarked that the mutations of *Oe. Reynoldsii* are not sufficiently characteristic in youth to admit of accurate classification, and that consequently every plant of each culture must be carried to maturity if it is to be certainly identified. With most of the other mutable species it is possible to discard many of the typical individuals which make up the bulk of the cultures without giving them garden space, since the young plants are as easily distinguished as the mature ones.

In marked contrast with *Oe. Reynoldsii*, all individuals of f. *typica* in the mass-mutant strain of *Oe. pratincola* seem capable of throwing the mutations characteristic of mass mutation in that species, and such individuals differ among themselves as widely as possible in degree of mutability. Moreover, in *Oe. pratincola* the number of abortive seeds seems to vary in approximately inverse proportion to the number of *typica* individuals obtained from the seeds. This fact might be adduced as an argument for considering the bad seeds as resulting from the abortion of *typica* zygotes. We do not wish to minimize this possibility but prefer for the present the hypothesis that the zygotes which fail to develop represent mutational types of excessively weak constitution.

It appears at present that mass mutation in *Oe. Reynoldsii* differs considerably from the similar process in *Oe. pratincola*, the chief difference being that in the former species the characteristic mutations are produced only by certain individuals of f. *typica* in which there is great seed sterility, whereas in the latter species any individual of f. *typica* belonging to the mass mutant strain may give rise to the characteristic mutations, the mutable individuals differing widely among themselves in mutability and seed sterility. The process is alike in both species in that the characteristic mutations occur only in strains some members of which are excessively mutable (*i. e.*, mass-mutant) and in that the characteristic mutations in both cases show matroclinic inheritance.

Before turning to the evidence in regard to matroclinic inheritance there is a further feature of seed abortion to which attention should be called. The germination data in Table I show clearly that mutations arising from highly infertile mass-mutant f. *typica* are not themselves excessively infertile. The degree of seed abortion is not nearly as great in the mutations as in the parent plant that produced them. Seeds of mass-mutant f. *typica* have given germinations varying from 2.3 to 5.1 percent. In striking contrast to this low viability, seeds of mut. *semialta* have given germinations of 88.7 percent and 62.0 percent; seeds of mut. *rigida*, 72.0 percent. The germinations recorded for mut. *bilonga* are much lower than the true value, because only green plants that survived were counted. This mutation has the curious characteristic of giving rise to progenies consisting of a mixture of green and yellow plants. The latter lack the capacity for chlorophyll production, and die shortly after the cotyledons unfold. The relative numbers of green and yellow plants have not yet been determined. Leaving yellow plants out of consideration, mut. *bilonga* has given progenies numbering 34.6 percent, 24.0 percent, and 25.9 percent of the number of seeds sown—well in excess of the viability of mass-mutant f. *typica*. Complete records have been kept for only one progeny of mut. *debilis*. This form is a weak dwarf, of which the seeds are much less viable than those of the other mutations. Only 7.5 percent of germinations were obtained. It must be remembered, however, that mut. *debilis*, the most sterile of the mutations, gives rise to mut. *bilonga*, a form showing a distinct increase in fertility over its parent. We can not doubt that in the case of the mutations seed sterility is in a large measure inversely proportional to the vegetative vigor of the parent plant.

The same explanation does not hold for the difference between the two kinds of f. *typica*, for vegetatively they are equally vigorous. May not the yellow seedlings which occur in progenies of mut. *bilonga* give a clew to an understanding of the situation? These yellow seedlings constitute a mutational type in which chlorophyll formation can not take place, and therefore a type which can not persist more than a few days after germination. It does not require a very great effort of the imagination to conceive of physiological defects that might originate by mutation and that might operate disadvantageously to the organism possessing them at an even earlier stage in the life cycle than failure to produce chlorophyll. May not the aborting seeds in the polymorphic progenies represent one or more physiologically defective classes of mutations, of which the zygotes are unable to develop into mature embryos? Pending cytological study of the abortive seeds, such a hypothesis seems to us much more plausible than the alternative hypothesis that they are *typica* zygotes, eliminated by some unknown selective process that leaves to develop the intrinsically weaker zygotes of the several mutational types.

Matroclinic Inheritance in the Mutation Crosses

In 1915 a complete series of mutation crosses was made, involving f. *typica* and the three well-known mutations. One parent plant of each form served for self-pollination and for crossing with the three other forms. Each cross was made reciprocally. Two of the twelve crosses, mut. *semialta* × mut. *debilis* and mut. *bilonga* × mut. *debilis*, failed, but the remaining ten were in varying degrees successful, and progenies of all were grown in 1916. The reader will find the four parent plants of these crosses indicated by asterisks in figure 1, and may determine by reference to Table I that all gave rise to uniform progenies in the following generation. It will be observed that the phenomenon of mass mutation had not occurred in the direct line of descent of the individual of f. *typica* chosen as a parent. Both the *semialta* and the *bilonga* parents belonged to first generation progenies from primary mutations (*i. e.*, mutations derived directly from f. *typica*, and not from one of the other mutations). The former type arises only as a primary mutation, but the latter is frequently derived as a secondary mutation from mut. *debilis*. The individual of mut. *debilis* used as a parent was an actual primary mutation in a polymorphic progeny, chosen because, at the time the other plants were in condition for crossing, the uniform first generation culture of mut. *debilis* did not contain a single plant on which enough flowers remained to suffice for all of the crosses. The detailed analysis of the mutation crosses is given in Table II.

In brief, the results of the mutation crosses are as follows:

typica × semialta → typica
typica × debilis → typica
typica × bilonga → typica + yellow twin
semialta × typica → semialta
semialta × bilonga → semialta
debilis × typica → debilis
debilis × semialta → debilis
debilis × bilonga → debilis + bilonga
bilonga × typica → bilonga + yellow twin
bilonga × semialta → bilonga + yellow twin

With one exception the scheme of inheritance is strictly matroclinic. The type of pollen used is immaterial, providing it does not come from mut. *bilonga*. All progenies which did not have mut. *bilonga* as the pollen parent were exactly the same as they would have been if the mother plant had been self-pollinated. The fact has already been mentioned that progenies of self-pollinated mut. *bilonga* consist of a mixture of green and yellow plants. Every cross into which mut. *bilonga* entered as the pistillate parent showed exactly the same mixture of green and yellow plants, of which the former developed as normal mut. *bilonga* and the latter died. It is obvious, however, that the crosses with mut. *bilonga* as pollen parent constitute a real exception to the prevalence of matroclinic inheritance in the mutation crosses.

In the case of mut. *debilis* × mut. *bilonga* the progeny contained both the maternal and the paternal types, the latter in such large numbers that it was not possible to view them as having arisen *de novo* by mutation from *debilis* eggs. Thus the progeny from the cross contained 18 plants of mut. *bilonga* out of a total of 47 plants. By way of contrast, the progeny of the pistillate parent, mut. *debilis*, self-pollinated, included only two individuals of mut. *bilonga* in a total of 62. Mut. *bilonga* was therefore roughly twelve times as frequent in the cross as in the progeny resulting from self-pollination, —a difference that one must ascribe to the pollen parent. Incidentally, it seems worth while to call attention to the fact, without attempt-

TABLE II

Summary of the Cultures of Mutation Crosses, Grown in 1916. The Parents of the Crosses are Indicated by Asterisks in Fig. 1, and the Composition of Progenies Derived from Them by Self-pollination May be Found in Table I

Progeny No.	Cross	♀ Parent	♂ Parent	Seeds Planted	Germination		Retained to Maturity	*Typica*	*Semi-alta*	*Debilis*	*Bilonga*	Other Mutations
					No.	%						
27	*typica* × *semialta*	89–53–1	89–3–13–4–16	1,021	861	84.3%	200	199	. .	. .	. .	1(#143)
28	*typica* × *debilis*	89–53–1	89–3–13–134	569	458	80.5%	200	200	. .	. .	. .	. .
29	*typica* × *bilonga*	89–53–1	89–3–53–22	840	162*	19.3%*	162	162	. .	. .	. .	Many*
30	*semialta* × *typica*	89–3–13–4–16	89–53–1	593	333	56.2%	200	. .	197	3	. .	. .
31	*semialta* × *bilonga*	89–3–13–4–16	89–3–53–22	783	221	28.2%	200	. .	189	1	10	. .
32	*debilis* × *typica*	89–3–13–134	89–53–1	184	14	7.6%	14	. .	. .	14	. .	. .
33	*debilis* × *semialta*	89–3–13–134	89–3–13–4–16	99	34	34%	34	. .	. .	34	. .	. .
34	*debilis* × *bilonga*	89–3–13–134	89–3–53–22	105	52	50.0%	47	. .	. .	29	18	. .
35	*bilonga* × *typica*	89–3–53–22	89–53–1	153	50	32.7%	50	. .	. .	. .	50	. .
36	*bilonga* × *semialta*	89–3–53–22	89–3–13–4–16	501	150	30.0%	140	. .	. .	. .	140	. .

* Seeds of the cross f. *typica* × mut. *bilonga* gave a mixed progeny of green and yellow plants, apparently in about equal numbers. The yellow plants died off rapidly and were not counted. The germination record therefore takes account of the green plants only.

ing to draw any conclusions from it, that seeds of mut. *debilis* were much less viable than those of the crosses into which this form entered as the pistillate parent.

The progeny obtained from the cross f. *typica* × mut. *bilonga* showed the influence of the pollen parent in the large number of yellow plants, the same, as far as superficial observation could indicate, as the yellow twin that appears in progenies from self-pollinated mut. *bilonga.* In view of the fact that the crosses *typica* × *bilonga* and *debilis* × *bilonga* both resulted in twin types, it is interesting that the third cross, *semialta* × *bilonga,* gave only plants of the maternal type, aside from a few which appear to be satisfactorily accounted for as derived from mutated gametes.

It will be observed from a scrutiny of Table II that in the foregoing discussion we have tacitly assumed that the sporadic occurrence of types in cultures where they would not necessarily be expected to occur was to be ascribed to mutation. We have made no special comment, for example, on the few individuals of mut. *debilis* that turned up in the progeny of *semialta* × *typica,* for the reason that mut. *semialta* always seems to produce some mutated gametes that give rise on fertilization to mut. *debilis.* Furthermore, there is every ground for the belief, on evidence furnished by the matroclinic progeny of the cross *debilis* × *typica,* that the few *debilis*-yielding eggs of *semialta* would give rise to mut. *debilis* quite regardless of the source of the male gametes, provided, of course, that the latter were not derived from mut. *bilonga,* the one form of the series that seems to give rise to more than one type of sperms.

To return to the case of the cross *semialta* × *bilonga,* we have seen that mut. *semialta* always produces some *debilis*-yielding eggs, and that the cross *debilis* × *bilonga* yields a progeny containing both parental types. Consequently we should expect that in the cross *semialta* × *bilonga* some of the mutated eggs would give rise to mut. *debilis* and some to mut. *bilonga,* whereas in a progeny resulting from self-pollination or from one of the other crosses the mutated eggs would be represented by mut. *debilis* alone. The results of the crosses realize this expectation. Although mut. *semialta,* whether self-pollinated or crossed, has always given rise to mut. *debilis,* it has never given mut. *bilonga* except in the case of the cross *semialta* × *bilonga.*

The results of the whole series of cultures are intelligible on the supposition that *Oe. Reynoldsii* is one of the mutable species to which

De Vries[9] would apply the term heterogamous. It has frequently been found that crosses of the Oenotheras differ strikingly according to the direction in which the cross is made. Often the reciprocal hybrids from the same two parent plants are as unlike as the parents themselves. De Vries has attributed such results to a difference in the hereditary qualities of the male and female gametes, and has suggested the term heterogamy for the condition of species in which such a differentiation of gametes is found. There is much unpublished evidence at hand which tends to show that heterogamy may exist in some species without a sharp restriction of either type of gamete to the eggs or sperms and on this account we shall use the term "heterogamy" with no implication that the non-equivalent gametes may not exist on both the male and female sides. The conception of heterogamy so modified as to apply to results that have been obtained in our experiments has been published[10] in advance of the data which suggested the modification.

Let us assume (1) that a heterogamous species such as *Oe. Reynoldsii* normally produces two types of non-equivalent gametes, which may be designated as α and β respectively; (2) that the α gametes carry most of the characters by which specific differentiation is effected; (3) that mutation occurs through the modification of α gametes, which thus become α', α'', α''', etc. Applying this conception to the particular case in hand, let us think of f. *typica* as the zygote $\alpha\beta$, mut. *semialta* as $\alpha'\beta$, mut. *debilis* as $\alpha''\beta$, and mut. *bilonga* as $\alpha'''\beta$. The conditions imposed by the results of the various crosses are satisfied if

f. *typica* → α eggs + β sperms,
mut. *semialta* → α' eggs + β sperms,
mut. *debilis* → α'' and β eggs + β sperms,
mut. *bilonga* → α''' and β eggs + α''' and β sperms.

Since the various forms are determined by the α gamete, all mutation crosses must of necessity show matroclinic inheritance, except those involving mut. *bilonga*, for this one form is the only member of the series that produces any male α gametes. In order to be functional, male α gametes must fuse with female β gametes, which are produced only by mut. *debilis*. Therefore the cross *debilis* × *bilonga* is the only one that yields both the maternal and paternal types. It will be remembered that mut. *debilis*, when self-pollinated, was marked by great seed sterility. This sterility was much reduced when pollen from one of the other forms was used, and the effect of foreign pollen was greatest of all when that of mut. *bilonga* was used. Doubtless several factors are concerned with the increase of fertility on crossing, but it seems not unwarranted to call attention to the fact that if our hypothesis were true such an increase would be expected, because good embryos would result from the fertilization of female β gametes by male α gametes. All the *bilonga* individuals in the mixed progeny from *debilis* × *bilonga* would be represented in a self-pollinated progeny by aborted seeds.

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[9] De Vries, Gruppenweise Artbildung, pp. 30–32.

[10] Bartlett, H. H., The Status of the Mutation Theory, with Especial Reference to Oenothera, Amer. Nat., **50**: 513–529. 1916.

On the whole, the facts point to the truth of the hypothesis of non-equivalent gametes. The facts to be explained are sufficiently orderly to demand more than a superficial criticism at the hands of those who see in the mutation phenomena merely evidence of Mendelian segregation. It seems to the writers that the work with *Oe. Reynoldsii* affords very convincing evidence of De Vriesian mutation.

Quantitative Evidence of Matroclinic Inheritance

Although no one who has had an opportunity to examine the mutation crosses has doubted the fact of matroclinic inheritance, it was of course essential to obtain quantitative data that would convince one of the accuracy of our observations. Leaves and capsules from self-pollinated and crossed progenies were therefore measured, both in order to establish the fact that the several forms differed widely from one another and to show that the mutation crosses resembled the pistillate parent. In most cases a large enough number of plants was at hand to give satisfactory data.

Mature stem leaves were measured from plants of all the pure strains and mutation crosses, five leaves being taken at the same part of the main stem from each plant as it came in the row, without selection. The leaf lengths are summarized in Table III, the widths in Table IV. The two tables are based upon the same material, but individual leaves were frequently imperfect, so that one or the other measurement could not be made. On this account the number of measurements does not always tally in the two tables. It is very clear that the modes of the variation curves lie very close together in the cases of all progenies having the same pistillate parent. There are some discrepancies, to be sure, the most notable being the failure of a closer

TABLE III

Leaf Lengths of Oe. Reynoldsii, its Mutations and Mutation Crosses

Length in Mm.	F. *Typica*					Mutation *Semialta*			Mutation *Debilis*					Mutation *Bilonga*			
	× *Typica*		× *Semi-alta* No. 27	× *De-bilis* No. 28	× *Bi-longa* No. 29	× *Semi-alta* No. 17	× *Ty-pica* No. 30	× *Bi-longa* No. 31	× *De-bilis* No. 19	× *Ty-pica* No. 32	× *Semi-alta* No. 33	× *Bilonga* No. 34		× *Bi-longa* No. 26	× *Ty-pica* No. 35	× *Semialta* No. 36	
	Pro-geny No. 8	No. 6										*Debilis*	*Bilonga*			*a*	*b*
30–39	..	..	..	..	..	..	..	..	..	2	..	..	..	..	..	..	..
40–49	..	..	..	..	..	..	..	..	1	**28**	1	..	..	..	..	..	..
50–59	..	..	..	..	..	..	..	..	12	**28**	12	14	1	..	..	..	..
60–69	..	..	..	..	..	..	..	..	60	11	**65**	**88**	11	16	1	5	9
70–79	..	..	..	..	..	2	..	2	**101**	..	23	72	31	69	43	38	35
80–89	..	..	..	..	..	21	..	21	37	..	..	10	**42**	**110**	**106**	75	**118**
90–99	..	..	..	..	..	49	13	47	1	..	..	..	19	30	76	**125**	110
100–109	..	..	..	..	..	59	22	66	..	..	..	..	9	1	28	68	16
110–119	6	..	..	..	1	**73**	52	118	..	..	..	..	5	..	..	13	6
120–129	5	..	..	..	1	35	70	**120**	..	..	..						
130–139	2	7	2	1	3	14	**83**	93	..	..	..						
140–149	19	40	20	7	17	3	41	34	..	..	..						
150–159	46	**68**	74	35	41	..	23	9	..	..	..						
160–169	**122**	38	**85**	72	78	..	5	1	..	..	..						
170–179	88	15	75	**119**	**92**	..	..	..	..	..	..						
180–189	14	4	25	65	47												
190–199	1	1	13	17	25												
200–209	..	..	5	1	9												
210–219	..	..	..	1	..												

TABLE IV

Leaf Widths of Oe. Reynoldsii, Its Mutations and Mutation Crosses

Width in Mm.	F. *Typica*					Mut. *Semialta*			Mut. *Debilis*					Mut. *Bilonga*			
	× *Typica*		× *Semi-alta* No. 27	× *De-bilis* No. 28	× *Bi-longa* No. 29	× *Semi-alta* No. 17	× *Ty-pica* No. 30	× *Bi-longa* No. 31	× *De-bilis* No. 19	× *Ty-pica* No. 32	× *Semi-alta* No. 33	× *Bilonga* No. 34		× *Bi-longa* No. 26	× *Ty-pica* No. 35	× *Semialta* No. 36	
	Progeny No. 8	No. 6										*Debilis*	*Bilonga*			*a*	*b*
10–11	..	..	..	..	..	..	..	..	..	..	2	2	..	..	..	..	..
12–13	..	..	..	..	..	..	..	..	19	13	13	16	..	5	..	1	..
14–15	..	..	..	..	..	..	..	..	**75**	**28**	**43**	58	..	8	1	0	4
16–17	..	..	..	..	..	..	..	..	**72**	22	36	**64**	5	32	5	15	21
18–19	..	..	..	..	..	..	..	..	36	6	6	30	31	52	49	55	70
20–21	..	..	..	..	..	..	..	1	8	..	..	13	**45**	**79**	**97**	93	111
22–23	..	..	..	..	..	3	..	12	2	..	..	1	22	28	49	**97**	76
24–25	..	..	..	..	..	15	3	17	..	..	..	..	9	15	38	50	19
26–27	1	..	..	..	..	30	3	39	..	..	..	..	2	7	12	3	1
28–29	0	..	..	..	..	42	7	55	..	..	..	..	..	1	1	5	..
30–31	6	1	1	..	..	**51**	15	87	..	..	..	..	..				
32–33	4	5	0	..	1	48	30	**89**	..	..	..	..	..				
34–35	4	11	4	..	1	39	32	88	..	..	..	..	..				
36–37	5	15	6	..	3	21	47	63	..	..	..	..	..				
38–39	10	21	16	4	5	19	**65**	37	..	..	..	..	..				
40–41	34	27	46	14	29	3	45	24	..	..	..	..	..				
42–43	66	**32**	**62**	37	45	0	26	7	..	..	..	..	..				
44–45	**71**	19	55	**98**	67	0	17	3	..	..	..	..	..				
46–47	42	16	45	88	**71**	0	13	1	..	..	..	..	..				
48–49	17	11	34	54	50	..	4	..	..	..	..	..	..				
50–51	1	2	21	21	27	..	1	..	..	..	..	..	..				
52–53	..	..	12	3	9	..	..	..	..	..	..	..	..				
54–55	..	..	3	1	6	..											
56–57	..	..	1	..	2	..											

agreement between *semialta* × *semialta* and *semialta* × *typica*, and, conversely, the unexpected closeness of the modes for *semialta*×*typica* and *typica* × *semialta*. It is believed, however, that the departures from the expected are all explained by environmental non-uniformity. The garden itself was relatively uniform, but the cultures were set out at different times, so that some of the plants were subjected to hot dry weather much sooner than others. The most rapid growth took place early in the season, with the result that the last plants to be transplanted were markedly the weakest. Without exception, the greater deviations from the measurements which would have been expected in matroclinic inheritance were correlated with the greater intervals between time of transplanting, and, conversely, the best agreements with expectation were found in the cases of cultures set out on the same day.

A valuable evidence of shifting of the mode attributable to difference in date of transplanting was quite accidentally obtained in the case of the cross *bilonga* × *semialta*. The culture had been partly transplanted at the close of the day, and the next morning the remainder was overlooked. It was set out a couple of weeks later. Leaves from the two lots were collected separately, and the data are given separately in Tables III and IV.

Considering the unfavorable experimental conditions, the measurements provide as good a demonstration of matroclinic inheritance as could reasonably be demanded. Moreover, it would be unfair not to emphasize the fact that in the one or two cases where the measurements might appear ambiguous, the plants were in general aspect true to the expected type. Thus the cross *semialta* × *typica* had the whole appearance of self-pollinated *semialta*, from which it differed only in being much more robust. Although we are of the opinion that environmental non-uniformity explains the difference, we shall carry out more carefully controlled experiments to see if cross-pollination produces any effect similar to the vigor of heterozygosis. Such an effect might conceivably be measurable if environmental non-uniformity were eliminated, but if it exists it is obviously not large enough to obscure the underlying phenomenon of matroclinic inheritance.

The data for capsule length in the mutation crosses are incomplete because of the fact that some of the cultures of 1916 were just coming into flower at the time of the first heavy frost and were destroyed. Before examining the data for the crosses, the reader should glance at

Table V, which shows the range of variation in capsule length in each of the four pure forms. The measurements were made in Maryland in 1915. In the cases of f. *typica* and mut. *semialta* the ten lowest normal capsules of the main inflorescence of each plant were used. The capsules of mut. *debilis* were taken from the *debilis* plants of a polymorphic progeny. They were collected at random, because very few inflorescences bore as many as ten good fruits. Capsules of mut. *bilonga* were measured from two different cultures, in order to demonstrate the essential identity of this mutation, whether obtained directly from f. *typica*, or as a secondary mutation from mut. *debilis*. The measurements prove the anticipated identity, or, if anything, give a false impression that the secondary mutation is stronger than the primary. This impression is due to the fact that at the time the measurements were made the secondarily derived mut. *bilonga* had been so short a time in flower that ten full-grown capsules could not be obtained from each inflorescence. Six fruits were therefore taken from each, and the greater average length which they show in comparison with primarily derived mut. *bilonga* is due to their lower position in the inflorescence. The progeny of primary mut. *bilonga* was arbitrarily divided into a class of weak plants and a class of strong. The capsules of these two classes, ten from each plant, were separately measured, and the results are recorded in Table V for each class separately and for the two classes combined. The discrepancy between the two classes was much less than was expected, and indicated clearly that the capsules respond less to environmental conditions than vegetative parts of the plant. Measurements of any other part would have shown a much more marked difference between the arbitrarily selected strong and weak plants.

On account of its relative independence of environmental factors the capsule length affords us a more conservative criterion of matroclinic inheritance than the leaf measurements which have already been considered. In this respect it seems to be similar to the character of flower size, which East[11] has found especially useful in his studies of inheritance of quantitative characters in Nicotiana. In *Nicotiana sylvestris* he found that adverse environmental conditions, which brought about a shortening of the leaf amounting to three fourths of its normal length, did not appreciably change the size of the flowers.

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[11] East, E. M., Inheritance of Flower Size in Crosses between Species of Nicotiana, Bot. Gaz. **55**: 177–188. 1913.

TABLE V

Length of Capsule in Oe. Reynoldsii and its Mutations

Length in Mm.	*Typica* Progeny No. 7	*Semialta* No. 16	*Debilis* No. 11	*Bilonga* from *Debilis* No. 23	*Bilonga* from *Typica*: Weak No. 25	*Bilonga* from *Typica*: Strong No. 25	*Bilonga* from *Typica*: Sum No. 25
14–15	....	....	7	....	....	....	....
16–17	....	....	38	....	....	....	....
18–19	....	....	86	....	....	....	....
20–21	....	6	**105**	....	....	....	....
22–23	....	43	**104**	....	....	....	....
24–25	....	97	84	....	....	....	....
26–27	....	**122**	51	....	....	....	....
28–29	....	94	31	....	....	....	....
30–31	4	57	12	....	....	....	....
32–33	21	21	6	....	....	....	....
34–35	65	13	3	....	....	....	....
36–37	165	2	....	....	....	....	....
38–39	**260**	1	....	....	....	....	....
40–41	223	1	....	....	....	....	....
42–43	82	....	....	1	....	....	....
44–45	10	....	....	0	3	....	3
46–47	....	....	....	4	14	5	19
48–49	....	....	....	8	24	18	42
50–51	....	....	....	49	41	54	95
52–53	....	....	....	87	**72**	87	**159**
54–55	....	....	....	122	64	**91**	**155**
56–57	....	....	....	130	53	89	142
58–59	....	....	....	**141**	21	60	81
60–61	....	....	....	110	11	49	60
62–63	....	....	....	71	7	31	38
64–65	....	....	....	36	6	21	27
66–67	....	....	....	18	3	5	8
68–69	....	....	....	5	1	6	7
70–71	....	....	....	3	....	3	3
72–73	....	....	....	1	....	0	0
74–75	....	....	....	....	....	1	1

Goodspeed and Clausen[12] have questioned East's conclusions, and have demonstrated beyond question that the flower size does respond to environmental changes, but their results really strengthen the contention, that, as compared with changes in other parts, the flowers are relatively little affected. Our own conclusion in regard to the fruits of Oenothera is that they respond in size to varying environment, but that the response is relatively much less than the response in height of plant or size of leaves, and that the character of capsule length is particularly significant as a criterion of matroclinic inheritance.

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[12] Goodspeed, T. H., and Clausen, R. E., Factors Influencing Flower Size in Nicotiana, with Special Reference to Questions of Inheritance, Amer. Journ. Bot. **2**: 332–374. 1915.

TABLE VI

Capsule Lengths of Oe. Reynoldsii f. typica and mut. semialta, and of Some of their Mutation Crosses

The capsules measured were the lowest five capsules from each of two secondary inflorescences from each plant; the progeny numbers refer to Tables I and II.

Length in Mm.	F. *Typica*				Mut. *Semialta*		
	× *Typica* Progeny No. 8	× *Semialta* No. 27	× *Debilis* No. 28	× *Bilonga* No. 29	× *Semialta* No. 27	× *Typica* No. 30	× *Bilonga* No. 31
16–17	....	....	....	....	3	2	3
18–19	....	....	....	....	5	17	14
20–21	....	....	....	....	30	59	27
22–23	....	....	....	....	56	99	37
24–25	1	....	3	1	**114**	**147**	**44**
26–27	3	....	5	12	97	124	36
28–29	18	....	23	11	86	84	29
30–31	44	4	46	28	45	31	10
32–33	79	16	73	54	12	3	7
34–35	**100**	39	87	71	2	4	2
36–37	86	61	**99**	90	....	....	1
38–39	48	**92**	82	**110**	....	....	....
40–41	13	77	37	43	....	....	....
42–43	6	50	14	26	....	....	....
44–45	2	41	6	14	....	....	....
46–47	....	17	5	4	....	....	....
48–49	....	9	....	1	....	....	....
50–51	....	4	....	....	....	....	....

Table V brings out clearly the fact that the forms of *Oe. Reynoldsii* differ distinctly from one another in capsule length. It is not, however, strictly comparable with Table VI. In the first place, the garden of 1915 was in Maryland, where climatic, cultural and soil conditions were unlike those in Michigan. In the second place, the early frost in 1916 overtook the plants before the inflorescence of the main stem was sufficiently mature to provide full-grown capsules. Since the inflorescences of the long basal branches had begun to flower several days earlier, their lower capsules were full-grown. The five lower capsules from two branches of each plant were measured. Thus each plant provided ten capsules, but they were from two lateral branches rather than from the main stem. The capsules of the terminal inflorescence of the main stem are usually slightly larger than any others, and on this account the modes in Table V ought to be higher than in Table VI, as indeed they are. Perhaps the difference is not as great as it would have been if the capsules of the lateral branches had not

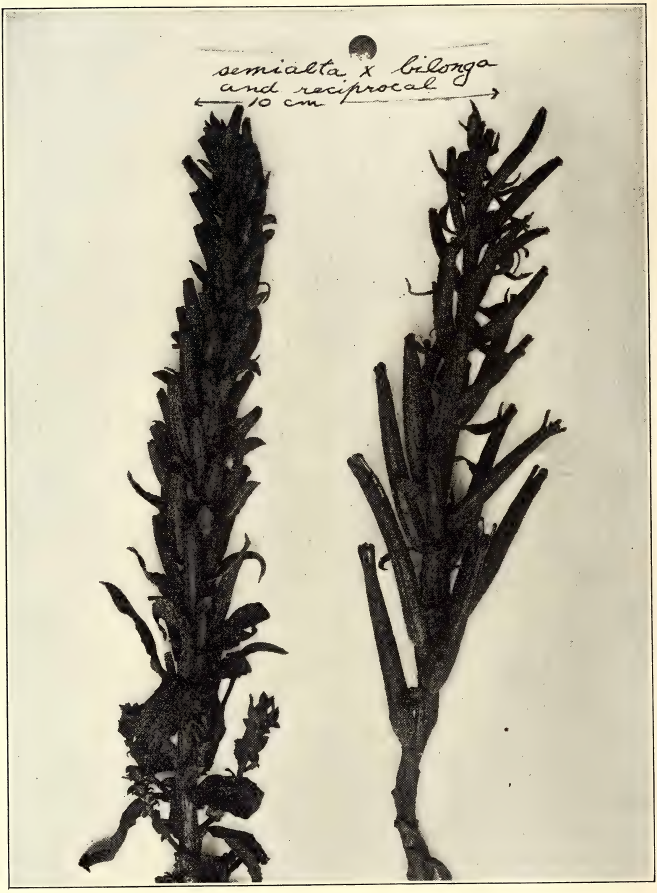
semialta x bilonga
and reciprocal
← 10 cm →

FIG. 2. Inflorescences showing mature fruits of the mutation crosses *semialta* × *bilonga* (left) and its reciprocal, *bilonga* × *semialta* (right). Each cross is matroclinic, and therefore the lengths of the fruits are in the ratio 1 : 2.

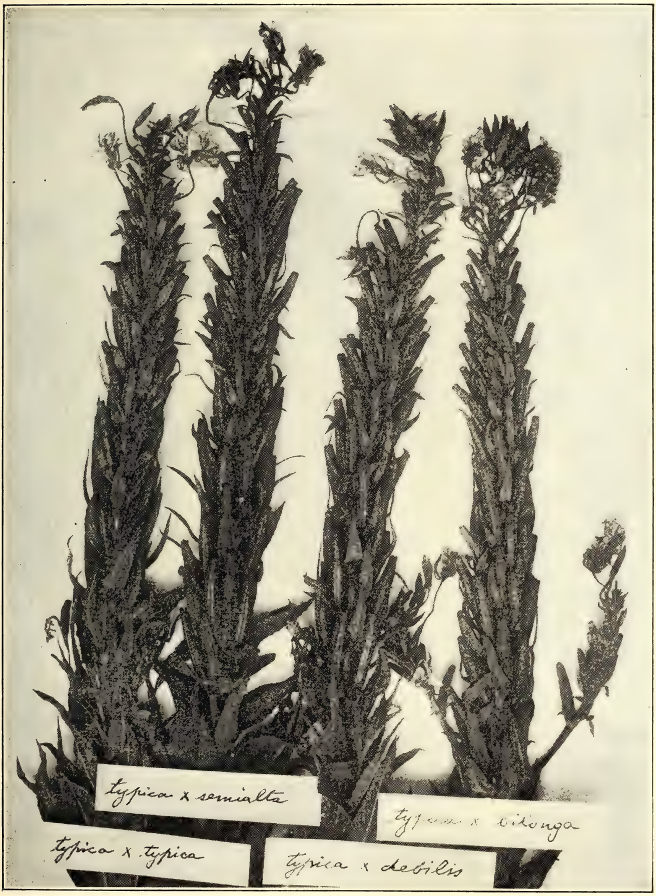
typica x semialta
typica x typica
typica x debilis
typica x bilonga

FIG. 3. The *typica* series of mutation crosses. The inflorescences are alike, resembling in each case the pistillate parent, *f. typica*. From left to right, *f. typica* (self-pollinated), *typica* × *semialta*, *typica* × *debilis*, and *typica* × *bilonga*.

had a lower average position in the inflorescence, for the lower capsules, if normally developed, are usually longer than those higher up.

Notwithstanding the difference in material, it is instructive to observe the close agreement between the two sets of measurements. The difference between the modal lengths for f. *typica* and mut. *semialta* in 1915 (Maryland) was just 12 mm., the same as the average difference between the *typica* series of cultures and the *semialta* series in 1916 (Michigan).

The capsule measurements give thoroughly satisfactory evidence of matroclinic inheritance. The data for the *semialta* series of cultures are particularly convincing. The modal lengths are the same for pure *semialta*, *semialta* × *typica*, and *semialta* × *bilonga*, being 25 mm. in each case. The ratio of the capsule lengths of the three pollen parents is 2:3:4, but the self-pollinated mut. *semialta* is just like the two crosses. Turning to the slightly less consistent data for the *typica* series, the evidence is hardly less satisfactory. In the cross *typica* × *debilis* the capsule length is actually slightly higher than in pure f. *typica*, in spite of the shorter capsule of the pollen parent. In the cross *typica* × *bilonga* the length is slightly greater than in self-pollinated f. *typica*, but that the difference is not significant is certain from the fact that the cross *typica* × *semialta* has capsules just as long. In the one case the pollen parent has a longer, in the other case a shorter, capsule than the pistillate parent, but the crosses are identical. The results of the capsule measurements, taken all in all, prove that matroclinic inheritance is the rule in the mutation crosses under consideration, and leads us to suspect that there may be such a thing as increased vigor due to cross-pollination, independently of factorial recombinations such as those that occur in Mendelian inheritance.

In the case of the very interesting dimorphic culture resulting from the cross *debilis* × *bilonga*, it is especially unfortunate that capsule measurements were not obtained, because the two forms differ so much from one another. That measurements would have fully substantiated the conclusions already drawn in regard to this cross is certain from the few precocious plants that matured before frost.

Figure 2 shows typical plants of the cross *semialta* × *bilonga* and its reciprocal. Of the latter there were too few plants that matured to make a series of measurements worth while. The fact of matroclinic inheritance is obvious from the photograph, however, for in *semialta* × *bilonga* the capsules are only half as long as in *bilonga*

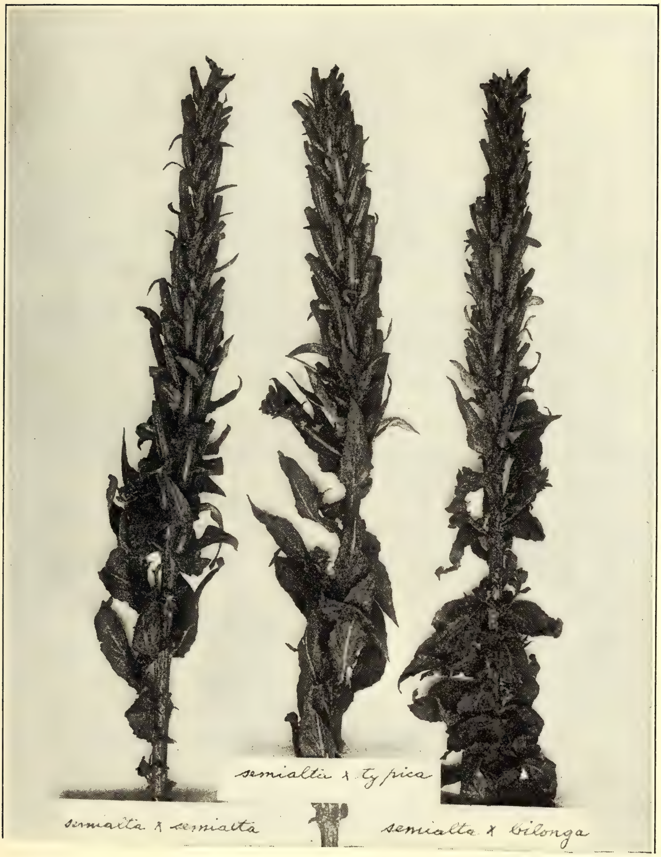
semialta x semialta
semialta x typica
semialta x bilonga

FIG. 4. The *semialta* series of mutation crosses. From left to right, mut. *semialta* (self-pollinated), *semialta* × *typica*, and *semialta* × *bilonga*.

× *semialta*. Figures 3 and 4 illustrate the *typica* series and the *semialta* series of crosses, respectively.

CONCLUSIONS

1. Mass mutation in *Oenothera Reynoldsii* consists in the production of inordinate numbers of mutations, belonging to several characteristic types, by certain mass-mutant individuals, which may be looked upon as having undergone a premutative modification.

2. Aside from their mutability, these mass-mutant individuals resemble normal f. *typica*. The production by them of a large number of abortive seeds may itself be looked upon as one of the manifestations of mutability.

3. The characteristic mutations form a series, each member of which may give rise to the succeeding member. Thus:
mut. *semialta* → mut. *debilis*,
mut. *debilis* → mut. *bilonga*.

4. Mut. *semialta* and mut. *debilis* appear to represent successive reduction stages in the mutation series. Mut. *bilonga*, on the contrary, marks an advance over the other members of the mutation series and also over f. *typica*.

5. With the exception of crosses involving mut. *bilonga*, the mutation crosses are matroclinic.

6. The cross *debilis* × *bilonga* gives a mixture of the two parental types.

7. The facts of inheritance are best explained by the hypothesis that two types of non-equivalent gametes, designated as α and β gametes, are normally produced.

8. The α gametes are usually eggs, and the β gametes sperms, but mut. *bilonga* produces both α and β sperms.

9. Mutation in *Oenothera Reynoldsii* consists in the modification in α gametes of factors that have no counterpart in the β gametes.

10. Since the sperms of f. *typica* are β gametes, mutations appear whenever a mutated α gamete is fertilized. They do not appear as a result of segregation.

DURATION OF LEAVES IN EVERGREENS

Vinnie A. Pease

While the duration of leaves in evergreens is not at all a new subject, very little systematic work seems to have been done toward determining durations for an extended list of evergreen species. This work was begun for the purpose of determining the leaf duration of the evergreen species of trees and shrubs in western Washington. It soon developed that the work would not be a mere cataloging of species with their accompanying leaf durations, since a very superficial examination of some of the coniferous evergreens growing under different conditions on the University campus, showed a wide but constant difference in the duration of their leaves. It was then decided to limit the species discussed to those growing under varying conditions that could be examined in the field, and to ascertain, if possible, the factors governing the duration of their leaves.

The Pacific northwest is peculiarly an evergreen region. Sargent (15) described the characteristic coniferous forests as the most luxuriant if not the most diversified on the continent. His report in the Tenth Census states that "Washington is covered with the heaviest continuous belt of forest growth in the United States. This magnificent coniferous forest extends over the slopes of the Cascade and Coast ranges, and occupies the entire drift plain surrounding the waters of Puget Sound." Evergreenness is not only characteristic of the forests, but is equally typical of the forest undergrowth, and of a large list of herbaceous species of the open fields. This is especially true of the Puget Sound region, in which the mild climate affords a practically continuous growing season. This may be one reason why many species elsewhere deciduous are here evergreen.

There are, in the state of Washington, according to Frye and Rigg (2), 76 species of woody evergreens, 24 of which are gymnosperms, and 52 angiosperms. In western Washington there are 52 species, 16 of which are gymnosperms, and 36 angiosperms. Of these the writer has studied the following 9 gymnosperms and 22 angiosperms:

Gymnosperms

1. *Abies grandis*
2. *Juniperus scopulorum*
3. *Picea sitchensis*
4. *Pinus contorta*
5. *Pinus monticola*
6. *Pseudotsuga taxifolia*
7. *Taxus brevifolia*
8. *Thuja plicata*
9. *Tsuga heterophylla*

Angiosperms

(*a*) *Transitional Forms.*—Those species which are deciduous under certain conditions and under others partly evergreen.

10. *Rhamnus purshiana*
11. *Vaccinium parvifolium*

(*b*) *Sub-evergreens.*—Those holding the leaves of one season only until the leaves of the next season are able to carry on the photosynthetic work of the plant. These species are not noticeably affected by external conditions.

12. *Arbutus menziesii*
13. *Ceanothus velutinus*
14. *Linnaea americana*
15. *Micromeria douglasii*
16. *Rubus laciniatus*
17. *Rubus pedatus*
18. *Rubus ursinus*

(*c*) *True Evergreens.*—Species which usually hold their leaves longer than the second season. These are noticeably affected by external conditions.

19. *Arctostaphylos tomentosa*
20. *Arctostaphylos uva-ursa*
21. *Berberis aquifolia*
22. *Berberis nervosa*
23. *Chimaphila menziesii*
24. *Chimaphila umbellata*
25. *Gaultheria shallon*
26. *Kalmia polifolia*
27. *Ledum groendlandicum*
28. *Oxycoccus oxycoccus intermedius*
29. *Pachistima myrsinites*
30. *Rhododendron californicum*
31. *Vaccinium ovatum*

Stark (16), in 1876, spoke of leaf duration as "not a new subject," yet at the same time declared his inability to find anything bearing on the subject in botanical literature. He made extensive observations on the native and introduced conifers in his large private grounds in the British Isles, and distinguished between true leaf fall, as shown in Taxus and Abies, and the shedding bodily of twigs (cladoptosis) as shown in Thuja, Pinus, and *Sequoia sempervirens.* He also remarked that old trees of Picea and Abies held their leaves for a shorter time than saplings.

Legget (10), in 1876, recognized the influence of climate on leaf duration especially in transitional forms.

Hoffman (7), for a series of years prior to 1878, carried on investigations with angiosperm evergreens in the Botanical Gardens at Giessen. He tied tinfoil tags to the petioles of six or eight leaves on a given plant and observed these individual leaves at stated intervals, reporting for several species the leaf duration in months. The method was too cumbersome to be applied on a very large scale, therefore his general conclusions seem hardly justified.

Kraus (8), in 1880, published on the duration of evergreen leaves. Unfortunately his work was not accessible to the writer.

Other writers, as Copeland (1) and Groom (4), also speak of leaf duration. Galloway (3), in 1896, enumerates various factors which may affect leaf duration in *Pinus virginiana;* but these references are all incidental, and mentioned in connection with other problems, or in general descriptions.

Sargent (13) (14) and Sudworth (17) in their descriptions of North American and Pacific Coast trees mention the leaf duration of many species, but their figures do not hold in some cases for the regions under discussion, and they give no estimates for other species which are quite common in this region.

The method of determining the age of a given leaf was simple. In those species having covered buds, the scars marking the boundaries of annual growth made it easy to count the years. In those species with naked buds, as *Thuja plicata* and *Juniperus scopulorum*, freehand sections were made through the twig at the base of the given leaf, and the annual growth-rings of the twig counted under the hand lens or low power of the compound microscope. This method was also used as a check in other doubtful cases.

When counting by means of terminal bud scars, the endeavor was to make counts of 100 twigs, but that was not always possible. In no case, however, did the count fall below 65. When counting by means of sections the attempt was made to obtain counts of 50 twigs. This was done in a majority of cases, and in no case did the count fall below 24. These counts were made in the field whenever possible; or the material was collected and carried to the laboratory, where the counts were made immediately, before handling dislodged leaves, or the unaccustomed dryness of the atmosphere caused them to fall. The counts were afterwards tabulated, and the tabulations placed on a percentage basis, the percent being calculated to the nearest whole number. Finally, curves were plotted from these data (*figs. 1–13*), the vertical axis representing the percent of twigs or branches examined which bore leaves persisting for the time in years denoted on the horizontal axis.

In making observations on gymnosperms, three chief points were considered on each twig or branch: (*a*) the year in which leaf fall commenced; (*b*) the year of maximum fall, that is, the time when the twigs were fully half bare; (*c*) the extreme duration of the last scattered leaves. In angiosperm species it was considered sufficient to make but one count for each twig or branch, and that to determine the age of the oldest persisting leaf.

The factors considered as having an influence on leaf duration were age of the tree, light, climate, and exposure to constant winds. When studying gymnosperms, observations were made on mature trees growing in the open and in close stands, as well as on saplings growing in the open and under the forest cover. In angiosperm species, observations were made from specimens growing in the open and under the forest cover. The observations included natural gymnosperm forest, partially cleared land, and second growth stands. These observations were made in the vicinity of Seattle, where the winds are not strong and the annual rainfall is about 36 inches.

In order to get contrasting climatic conditions, the writer spent the summer of 1915 at the Puget Sound Marine Station at Friday Harbor on San Juan Island, Washington. This island is sheltered by the Olympic Mountains, leaving the island an annual rainfall of less than 25 inches. The south slopes of the island are wind-swept, the trees having the characteristic one-sided form common to such regions. On this island the Seattle observations were repeated. Also observations were made to see if leaf duration varied in the same species on the leeward and windward slopes.

Several peat bogs in the vicinity of Seattle gave opportunity also to observe the effect of bog habitat on leaf duration. The observations were made partly at the bog one-half mile east of Ronald, Washington; partly at the Mud Lake bog, near the west shore of Lake Washington at 65th St., Seattle.

Since leaf duration varies with the conditions under which the plant is growing, and since these conditions are matters of general observation rather than of accurate measurements, it follows that the results are general. The longest durations are for the poorest combination of conditions; the shortest duration for the best combination of conditions; and the average duration merely the average of these conditions as nearly as could be ascertained from all the observations made. Mere general observation of the external and internal conditions of tree are not sufficiently accurate to enable one to predict with certainty just what one will find in a given tree.

1. *Abies grandis* Lindl. Shortest leaf duration observed, 2 years; average, 4–10 years; extreme, 14 years. All observations were made in the San Juan Islands, since the species is rare in the vicinity of Seattle. Old trees have a longer leaf duration than do saplings; shade tends to increase leaf duration; the leaves of wind-swept trees have a shorter duration than those of protected trees (*figs*. 9, 10).

2. *Juniperus scopulorum* Sarg. Shortest duration of green color observed, 1 year; average, 2–3 years; extreme, 4 years. The leaves, however, persist after turning brown. This results in the following: shortest leaf duration, 3 years; average, 4–6 years; extreme, 14 years. West of the Cascades this species occurs at low altitudes only in arid regions. It is quite common in the San Juan Islands. Two distinct types of leaves are found. The juvenile type, which are long, awl-shaped, and spreading, have a shorter duration than the adult, overlapping scale-like type. In all cases observed, the leaves lost their green color from 1–4 years before they fell, and were then gradually sloughed off.

3. *Picea sitchensis* Traut. & May. Shortest leaf duration observed, 2 years; average, 9–11 years; extreme, 18 years. In the vicinity of Seattle this species was observed only in peat bogs. In the San Juan Islands the trees observed stand at the head of a salt marsh which extends up a creek bed from False Bay. Mature trees in ordinary soil were not available and no saplings were observed, so that the results given are by no means complete.

4. *Pinus contorta* Dougl. Shortest leaf duration observed, 2 years; average, 4–6 years; extreme, 9 years. Leaf duration reported by Sargent (13), (14), 7–8 years; by Sudworth (17), 6–8 years. In the San Juan Islands, saplings in the open, and mature windswept trees, showed the shortest leaf duration; mature trees, protected from the wind, the longest duration. Trees introduced on the University campus showed the shortest duration observed. Sudworth states that "long persistence appears to belong more to young trees," but the writer found the opposite to be true.

5. *Pinus monticola* Dougl. Shortest leaf duration observed, 1 year;

average, 3–4 years; extreme, 6 years. Leaf duration reported by Sargent (13), 3–4 years. This species, found commonly in the Puget Sound region in peat bogs, showed the shortest duration of any of the gymnosperms studied. Mature trees show a tendency to hold their leaves longer than do saplings.

6. *Pseudotsuga taxifolia* Britton. Shortest leaf duration observed, 1 year; average, 3–9 years; extreme, 16 years. Leaf duration reported by Groom (4), Sargent (14) and Sudworth (17), about 8 years; by Ward (19), 6–7 years. Observations showed that saplings have a much shorter leaf duration than do mature trees; trees in the open have a much shorter leaf duration than those in the shade; wind-swept trees have a short leaf duration; a dry climate increases leaf duration; a peat bog habitat increases the duration of leaves in saplings to a greater degree than does a dry climate. No observations were made on mature trees in peat bogs. A winter season of unusual severity, such as that experienced by the Pacific northwest in January and February, 1916, when snow lay on the branches for several weeks, seriously affects the duration of the leaves. Thirty-eight percent of the branches examined showed partial loss of the leaves of the preceding season's growth, whereas no such loss was observed on the same trees during the same period of the preceding year. It was noted also that, in specimens of *Pseudotsuga taxifolia* growing in dense shade, the annual thickening of the trunks was very slight, the leafy twigs were very slender, and the needles small and comparatively few on a year's growth (*figs.* 1–5).

7. *Taxus brevifolia* Nutt. Shortest leaf duration observed, 2 years; average, 5–12 years; extreme, 23 years. Leaf duration reported by Sargent (14), 4–5 years; by Sudworth (17), 6–9 years.

A summary of the effects of varying external conditions cannot be given since not enough data could be secured. However, in ordinary conditions of moisture for the Puget Sound region, and in densely shaded locations in the drier climate of the San Juan Islands, the duration of leaves has been found to be much greater than previously supposed.

8. *Thuja plicata* Donn. Shortest duration of green color, 1 year; average, 2–5 years; extreme, 7 years. Since the leaves persist after losing their color the duration is longer than given above. Observations resulted in the following: shortest leaf duration observed, 3 years; average, 4–7 years; extreme, 12 years. Leaf duration reported by

Sargent (14) and Sudworth (17), about 3 years. Observations seem to indicate that the leaves of mature trees have a greater duration than those of saplings; that leaves in the shade have a greater duration than those in the open; that a dry climate seems to prolong the duration of the leaves; that a bog habitat has the same effect as a dry climate. Leaves remain on the tree for at least two or three years after losing their green color, and then are gradually sloughed off by the increase in size of the twig. Sudworth and Sargent also agree in saying that the lateral branchlets, which are shed entire, fall in their second year. The writer found that the duration of lateral branchlets also varies with habitat. Full data were not taken, but observations showed that under typical moisture conditions their duration was 2 to 3 years, while in bog specimens they persisted 4, 5 or 6 years (*figs.* 11, 12).

9. *Tsuga heterophylla* Sarg. Shortest leaf duration observed, 2 years; average, 4–7 years; extreme, 12 years. In general, mature trees show a greater leaf duration than do saplings under the same conditions of light and moisture. However, the shaded saplings observed in the vicinity of Seattle showed a greater leaf duration than that of mature trees growing under the same conditions. The saplings observed grew on fallen logs in dense shade under the parent trees, and had grown very slowly. Specimens which showed 20 annual growth-rings were less than a meter high, and no thicker than an ordinary lead pencil. The linear growth per year in many of the twigs examined was less than a centimeter, the needles on each year's growth were few in number, and the individual leaves were very small. There may be some correlation between this extreme slowness of growth and the increased duration of the leaves. Saplings in a moist climate show a longer leaf duration than saplings in a dry climate, while the converse is true for mature trees.

Bog saplings, observed in the peat bog at Ronald, Washington, were dwarfed and stunted in their growth to a much greater extent than the shaded saplings previously described. As determined by counting the annual rings under the low power of the compound microscope, these saplings ranged in age from 5 to 32 years. They were from 17 to 60 cm. high, but the height was not proportional to the age. Both lateral and terminal shoots averaged less than a centimeter per year in linear growth; and a year's growth in many cases comprised from 6 to 10 needles, which were much below normal in size. The leaf durations in these bog saplings show a remarkable feature, which was not observed in the case of any other species examined, under any condition. All three curves, that is, for beginning of leaf fall, for maximum leaf fall, and for extreme duration, show two maxima, the first occurring in the fourth year in all three cases, and the second in the sixth and seventh. This is probably due to variations in the toxicity of the bog water in different parts of the bog (13). Mature specimens from the bog also showed slow growth and small needles, but the duration curves were normal and the maxima lay between the two sets of maxima in the curves of the saplings. Peat bog specimens, both saplings and mature, show an increased leaf duration over specimens growing in the open in ordinary soil, the duration more nearly approximating that of specimens from a drier climate (*figs*. 6–8).

10. *Rhamnus purshiana* DC. Sudworth says that "in its northern habitat the thin large leaves are shed regularly in the autumn, while in the drier southern distribution to and through central California, the leaves, which are smaller, thicker, and somewhat leathery, often persist more or less during late autumn and winter." Frye and Rigg (2) state that the leaves are "deciduous except occasionally on very young plants." Sargent (14) says that "in Washington and Oregon the leaves fall late in November, while farther south and near the California coast they remain on the branches almost all winter, or until the following spring." The writer has found that not only do seedlings retain their leaves in the Puget Sound region, but that trees in moist rich humus under the forest cover, up to ten years old, may retain at least a part of their leaves well on into May, when the new season's leaves are fully expanded; and these persistent leaves seem not to differ in size or texture from those shed in the fall.

11. *Vaccinium parvifolium* J. E. Smith. The small plants which have germinated on fallen logs under the forest cover are almost invariably evergreen. The slender branches which arise from the root crowns of older shrubs also bear leaves which persist from one to several seasons. It was thought at first that evergreenness was confined to branches near the ground, but later several specimens were found which bore evergreen leaves from 1 to 2 meters above the ground, on the upper branches. Two distinguishing characteristics present themselves in regard to these evergreen leaves:

(*a*) The leaves are usually much smaller than the ordinary deciduous leaves, and are borne on very slender, slow-growing branches.

These branches attain a growth usually of less than 5 centimeters in a season, and may bear no more than 3 leaves on a season's growth. However, leaves of 3 or 4 years' growth have been found which were from 20 to 30 mm. in length, while the species description, Frye and Rigg (2), gives leaves 6 to 17 mm. long.

(*b*) While evergreen leaves are quite common, they are not usual on mature shrubs, and there seem to be no definite external factors which will explain their appearance or non-appearance. At best, only a few branches bear evergreen leaves. Also, of two shrubs of approximately the same age, growing under apparently the same conditions, and standing only 3 or 4 meters apart, one may be entirely devoid of leaves and the other have several branches bearing leaves of 3, 4, 5 or even 6 years' duration. The extreme duration observed was 6 years.

12. *Arbutus menziesii* Pursh. Observations were made on the campus of the University of Washington. The leaves begin to fall early in June of their second year. Many of the trees put on a second growth late in the summer, whose leaves are somewhat smaller and lighter in color than the normal spring leaves, and this gives the appearance of two seasons' growth. During the extreme and unusual cold weather of the past winter, many of the spring leaves were killed by frost while the late summer leaves seemed to be scarcely affected. This enhances still more the appearance of two seasons' growth.

13. *Ceanothus velutinus* Dougl. Like *Arbutus menziesii*, this normally holds the leaves of one season only until those of the succeeding season are fairly matured; that is, for a period of about 15 months.

14. *Linnaea americana*, Forbes. This trailing vine, as a rule, does not drop its leaves, but the leaves simply decay while attached, as they lie against the damp moss or already decaying leaves of the substratum. They persist throughout the winter, and in many cases until after the flowering season in the spring.

15. *Micromeria douglasii*, Benth. The same condition is found in this as in *Linnaea americana*.

16. *Rubus laciniatus* Willd. This plant has escaped from cultivation, and is commonly known as the "evergreen blackberry." Some leaves persist at least until after the flowering season.

17. *Rubus ursinus* Schlecht. & Cham. This is common on logged-off lands; according to Frye and Rigg (2) it is evergreen only in western Washington.

18. *Rubus pedatus* J. E. Smith. The writer found a single specimen, and that bore leaves of two seasons' growth.

19. *Arctostaphylos tomentosa* Dougl. The writer had access to only one specimen, a shrub which has stood for several years in the northwest angle of a 3-story building on the University campus. This showed a leaf duration of 4, 5 and 6 years on various branches.

20. *Arctostaphylos uva-ursa* Spreng. Shortest leaf duration observed, 2 years; average, 3 years; extreme, 5 years.

21. *Berberis aquifolium* Pursh. Shortest leaf duration observed, 1 year; average, 2–4 years; extreme, 6 years. Not found usually in shaded situations. A dry climate shortens its leaf duration.

22. *Berberis nervosa* Pursh. Shortest leaf duration observed, 2 years; average, 3–4 years; extreme, 8 years. Plants growing in the shade show a longer leaf duration than those growing in the open. A dry climate accents the difference in duration between leaves in the open and those in the shade.

23. *Chimaphila menziesii* Spreng. Shortest leaf duration observed, 2 years; average, 4–5 years; extreme, 8 years. This species was found only in a limited area on San Juan Island.

24. *Chimaphila umbellata* Nutt. Shortest leaf duration observed, 1 year; average, 2–4 years; extreme, 7 years. A dry climate tends to increase its leaf duration.

25. *Gaultheria shallon* Pursh. Shortest leaf duration observed, 1 year; average, 2–4 years; extreme, 6 years. Shade plants under typical moisture conditions have a shorter leaf duration than plants in the open, while under dry conditions plants in the open have the shorter leaf duration. Plants growing in sphagnum about the margins of peat bogs resemble in growth-habit plants growing in the open in ordinary soil, but have a decided tendency toward shorter leaf duration.

26. *Kalmia polifolia* Wang. Shortest leaf duration observed, 1 year; average, 2 years; extreme, 3 years. In contrast to Ledum, shaded plants showed a tendency to shorter leaf duration, and plants which had been growing for several years in the experimental gardens were entirely bare of leaves when observed in December.

27. *Ledum groenlandicum* Oeder. Shortest leaf duration observed, 1 year; average, 2–4 years; extreme, 5 years. Plants in an open peat bog showed the shortest leaf duration. Plants which had been transferred to the experimental gardens of the university campus several years ago showed a marked tendency to increased leaf duration.

Plants growing in the shade about the borders of the bog were much modified, being much taller; and with leaves larger, thinner, less revolute, and less densely clothed with hairs on the under surface. These leaves were of much longer duration.

28. *Oxycoccus oxycoccus intermedius* Piper. Shortest leaf duration observed, 1 year; average, 2–3 years; extreme, 4 years. Plants partially shaded by the taller growth of Ledum about the hemlock hillocks showed increased leaf duration.

29. *Pachistima myrsinites* Raf. Shortest leaf duration observed, 2 years; average, 3–4 years; extreme, 8 years. This species was observed only in the San Juan Islands. Plants growing in exposed locations on the windward side of the islands had a shorter leaf duration than those on the leeward side.

30. *Rhododendron californicum* Hook. Shortest leaf duration observed, 1 year; average, 2 years; extreme, 3 years. This plant was observed only on the university campus, where it is used extensively as an ornamental shrub.

31. *Vaccinium ovatum* Pursh. Shortest leaf duration observed, 2 years; average, 2–4 years; extreme, 7 years. Plants in the shade show a decided increase in leaf duration (*figs.* 13).

It has already been noted that in many of the gymnosperms growing under adverse conditions, that is, in dense shade or in peat bogs, leaves are smaller and fewer in number on a year's growth than on specimens of the same species growing under more favorable conditions. While the tendency is not so marked in all cases the same difference in size was noted between the leaves of mature trees and those of saplings, mature trees ordinarily having smaller leaves than those of saplings.

Kraus (9) observed that the length and vigor not only of the growing shoots but also of the needles vary in different seasons; and Reinke (12) demonstrated that in transplanted evergreens the needles formed during the growing season immediately following the transplanting are conspicuously shorter than those formed during either the preceding or the following season. This was afterward confirmed by Copeland (1), who measured the needles on transplanted evergreens on the campus of Indiana University. Former observations are thus extended to include the variation in size of leaves on trees of the same species of different age, or growing in different habitats.

Groom (5) observed that though the individual leaf is small, the aggregate leaf surface of the conifer often greatly exceeds that of the dicotyledonous tree; and Copeland (1) in his study on the size of evergreen needles found that in abnormal years, when the leaves are small, "the number of needles compensates the plant for their lack of size, sometimes furnishing an even greater surface of leaf than is borne on the normal year's growth of stem." Following the same line of thought, it may be that the longer duration of leaves on mature trees, or on trees growing under adverse conditions, which is correlated with a decrease in size, tends to keep up the total leaf area. With longer duration and smaller leaves in dense shade as compared with open situations, increased duration may be correlated with two factors. Reduced size of the individual leaf, and reduced photosynthetic activity, due to diminished light intensity, are both compensated by an increased number of leaves; and increased leaf duration would furnish this increase in the number of leaves.

In all angiosperm forms which were examined, both in the open and in the shade, the leaves on shaded plants were much larger than those on plants exposed to direct sunlight; and with the exception of *Gaultheria shallon* growing in the typical climatic conditions of the vicinity of Seattle, plants in the shade held their leaves longer than those in the open. Hasselbring (6), in commenting on his experiments with Cuban tobacco grown under a cheese-cloth shade, states that "the reduction in photosynthesis in the shade leaves was compensated by an increase in leaf area, so that the production was not diminished." In various species under discussion, it is quite possible that the increase in photosynthetic area, which compensates the decrease in light intensity, is due not only to the increased size of the leaves but also to their increased duration.

CONCLUSIONS

1. Leaf duration varies widely among the different evergreen species, ranging from *Rhamnus purshiana*, which in young plants sometimes holds part of the leaves of one season until those of the next season are mature, to *Taxus brevifolia*, which has an extreme leaf duration of 23 years.

2. Leaf duration varies widely in individuals of the same species of different age or growing in different habitats: (*a*) Saplings have a shorter leaf duration than mature trees in the same habitat. (*b*) Trees or shrubs growing in the open have a shorter leaf duration than those of similar age in the shade. (*c*) Trees or shrubs on a windward coast have a shorter leaf duration than those on a leeward coast. (*d*) Gym-

nosperms in a moist climate have a shorter leaf duration than those in a drier climate. (*e*) A peat bog habitat has an effect similar to a dry climate.

3. Those factors which cause slowness of growth, and thus only a slight increase in diameter of the axis, are accompanied by an increased duration of the leaves.

4. Under the same climatic conditions, those factors which cause an increase in transpiration are accompanied by a decrease in leaf duration, and thus by a decrease in the transpiring surface.

5. Those factors which cause a decrease in photosynthetic activity are accompanied by an increase in leaf duration, and thus by an increase in the photosynthetic area.

6. It is quite possible that the variations in leaf duration in a given species may be due to differences in transpiration or photosynthetic activity, caused by difference in age or habitat.

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EXPLANATION OF FIGURES 1-13

Horizontal figures indicate years; vertical figures indicate number of specimens. Unless otherwise stated, ... is curve showing beginning of leaf fall; ——is curve showing greatest leaf fall; - - - - is curve showing extreme duration of leaves.

FIG. 1. *Pseudotsuga taxifolia*, on San Juan Island; mature trees, in the open, on leeward slope.

FIG. 2. *Pseudotsuga taxifolia*, at Seattle; sapling, in the open.

FIG. 3. *Pseudotsuga taxifolia*, at Seattle; mature tree, in the open, after unusually cold weather.

FIG. 4. *Pseudotsuga taxifolia*, on San Juan Island; mature tree, in the open, on windward slope.

FIG. 5. *Pseudotsuga taxifolia*, at Seattle; mature tree, in the open.

FIG. 6. *Tsuga heterophylla*, at Seattle; mature tree, in peat bog.

FIG. 7. *Tsuga heterophylla*, on San Juan Island; mature tree, in the open.

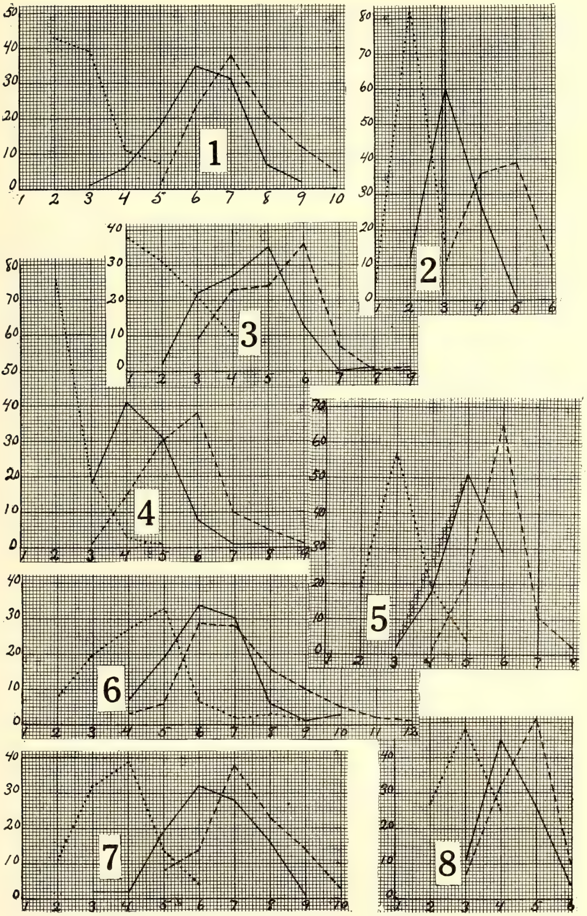
FIG. 8. *Tsuga heterophylla*, at Seattle; mature tree, in the open.

FIG. 9. *Abies grandis*, on San Juan Island; sapling, in the shade.

FIG. 10. *Abies grandis*, on San Juan Island; sapling, in the open.

FIGS. 11 AND 12. *Thuja plicata*, on San Juan Island; mature trees; Fig. 11 in the shade, Fig. 12 in the open. ...... is curve of loss of green color; —— is curve of beginning of leaf fall; - - - - is curve of extreme leaf duration.

FIG. 13. *Vaccinium ovatum*, at Seattle. —— is curve of extreme leaf duration in shade; - - - - is curve of extreme leaf duration in open.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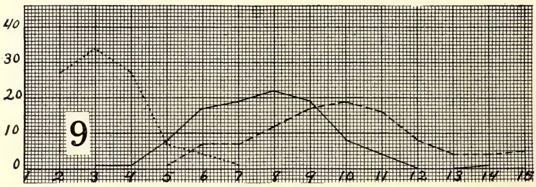
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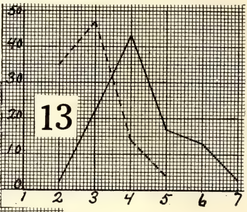
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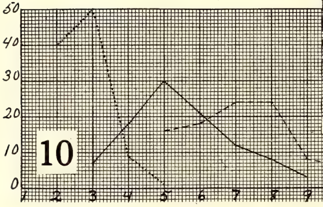
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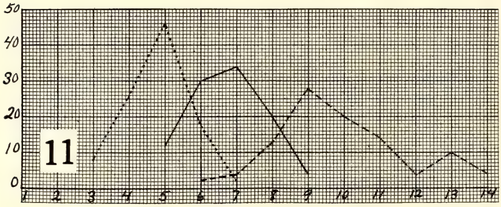
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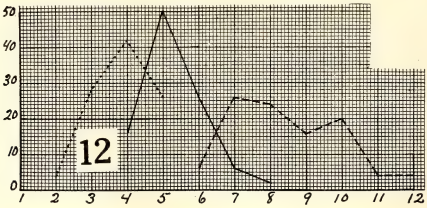
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THE RELATION BETWEEN EVAPORATION AND PLANT SUCCESSION IN A GIVEN AREA[1]

Frank C. Gates[2]

As a result of an investigation into the relative amounts of evaporation from the chamaephytic or ground layer of certain genetically connected, adjoining plant associations at Havana, Illinois, during the summer of 1910, Gleason and Gates (1) concluded: "that successions between associations are not caused by any conditions of evaporation." In conclusion to a much more extensive series of investigations, bearing on the same subject, Fuller (2) concludes: "the decreased rate of evaporation . . . is the direct cause of successions between different associations." Weaver (3) concludes: "A study of the differences of the rates of evaporation in the various plant formations and associations shows that these differences are sufficient to be important factors in causing succession, at least through the earlier stages, where light values are usually high."

Each investigation dealt with neighboring associations in a limited area, thereby accentuating the action of local factors and minimizing the obscuring interference of climatic factors. An inspection of the pertinent data obtained in each of these investigations shows that they are similar; yet diametrically opposite conclusions are drawn.

To obtain new data on the relationship between evaporation and plant succession, three series of experiments were carried on during the summers of 1915 and 1916, at the University of Michigan Biological Station at Douglas Lake, Michigan. During 1915, twenty-six standardized Livingston atmometers were employed for a period of 40 days, inclusive of the time of maximum evaporation during the year. In 1916, sixteen newly standardized instruments were employed during varying periods inclusive of the severest summer evaporation in years. Each instrument was set up in close proximity to certain plants. The experimentation and the calculation of the results to a standard basis followed the normal methods used for such work.

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[1] Contribution from the University of Michigan Biological Station at Douglas Lake, Michigan, No. 41.

[2] Owing to the press of duties attendant upon the establishment of the University of Michigan Botanical Garden, Dr. H. A. Gleason was unable to collaborate, as planned.

The object of this experimentation was the determination of the relationship between evaporation and plant succession in a local area. Douglas Lake region presents an admirable opportunity for such experimentation. A detailed discussion of the vegetation of the area will be found in Gates (4). A brief resumé of the pertinent facts is as follows: Aside from a few small associations, local along streams and around lakes, the vegetation of the region falls readily into three divisions, each characterizing a soil type. Bog associations, particularly the Chamaedaphne, Larix, Picea and Thuja associations, occupy the low wet soil. The sandy uplands were dominated by the pine association—now, following lumbering and fire, largely replaced by the aspen association. Clayey soil on the uplands is occupied by the hardwood or beech-maple association, except where it has been destroyed by lumbering or fire.

Experiments were carried on separately with the vegetation of each soil type. The Thuja association, chosen for the bog experimentation, is typically composed of a large number of trees of *Thuja occidentalis*, growing close together. The ground vegetation in a dense patch of Thuja is virtually nil. In open places, as along roads and trails, ericads and ericad-like plants are conspicuous. A few of the most abundant species are *Ledum groenlandicum*, *Streptopus amplexifolius*, *Moneses uniflora*, *Pirola asarifolia incarnata*, *Mitella nuda*, *Rubus triflorus*, *Cornus canadensis*, *Carex* spp., *Habenaria obtusata*, *Chamaedaphne calyculata* and *Vaccinium oxycoccus*.

The pine type—once represented by *Pinus strobus* and *Pinus resinosa*, now by scattering seedlings, small trees, and a few old trees of the same species mixed in with the aspen association—was investigated during 1915. At least 96 percent of the trees in the aspen association belong to the following four species: *Populus tremuloides*, *Populus grandidentata*, *Betula alba papyrifera*, and *Prunus pennsylvanica*. Among the higher shrubs are *Salix rostrata*, *Rhus glabra*, and *Viburnum acerifolium;* among the lower shrubs, *Diervilla lonicera* (which is frequently exceedingly abundant), *Vaccinium pennsylvanicum*, *Gaultheria procumbens*, *Rubus idaeus aculeatissimus*, and *Rubus allegheniensis* are quite common. The fern, *Pteris aquilina*, is frequently more abundant than any of the shrubs. With the shrubs are seedlings and small trees of *Quercus rubra*, *Acer rubrum*, *Acer sac-*

charum, *Fagus grandifolia*, *Tilia americana*, *Pinus resinosa*, and *Pinus strobus*. Among the herbaceous species are several grasses (*Panicum xanthophysum*, *Danthonia spicata*, *Poa pratensis*, *Agrostis hiemalis*, *Agrostis alba*, and *Oryzopsis asperifolia*), a very few sedges, and other plants, such as, *Convolvulus spithamaeus*, *Aster laevis*, *Hieracium scabrum*, *Hieracium venosum*, *Solidago canadensis*, *Melampyrum lineare*, *Fragaria virginiana*, *Smilacina stellata*, besides such common weeds as, *Erigeron canadensis*, *Rumex acetosella*, *Lepidium virginicum*, *Epilobium*

No.	Location. Hardwood Series.	Cc. in 40-Day Period.
1.	Cutover in 1914–15.	590
2.	Cutover in 1914–15.	561
3.	Open place in old cut.	453
4.	Cutover in 1913–14.	425
5.	Cutover in 1913–14.	416
6.	New margin of woods.	378
7.	Within margin 90 meters.	240
8.	Pine Point hardwoods.	210
9.	Opening in dense woods.	187
10.	Within dense Aspen thicket.	179
11.	Dense hardwoods.	175
12.	Very dense hardwoods.	147
13.	Burntover hardwoods near Bryant's.	473
14.	Bare ground near laboratory.	524
15.	Burntover pine land near Bryant's.	513
	Pine in Aspen Series.	
16.	Exposed place in upper flat.	347
17.	Exposed place on hill.	321
18.	Exposed place in lower flat.	315
19.	Exposed place on hill.	310
20.	Aspens in lower flat.	288
21.	Crest of slope in Aspens.	238
22.	Aspens in upper flat.	198
23.	Aspens in upper flat.	197
24.	Aspens in middle flat.	196
25.	Aspens in middle flat.	187
26.	Foot of slope in Aspens.	178

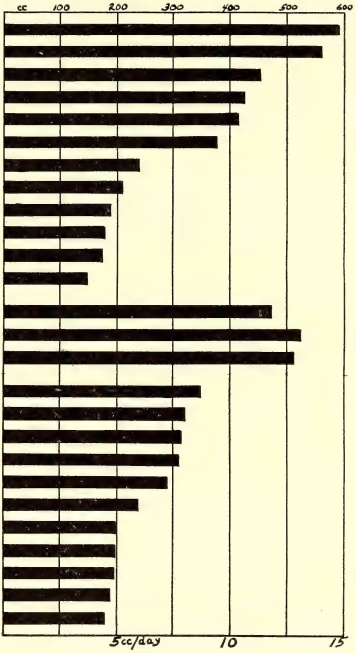

FIG. 1. Diagram showing the total evaporation and the rate per day for 40 days (July 10 to August 19, 1915) from different stations in hardwood and pine land in the vicinity of Douglas Lake, Michigan.

angustifolium, and *Erigeron ramosus*. Overtopping all other vegetation are a few scattered giant trees of *Pinus strobus* and *Pinus resinosa*.

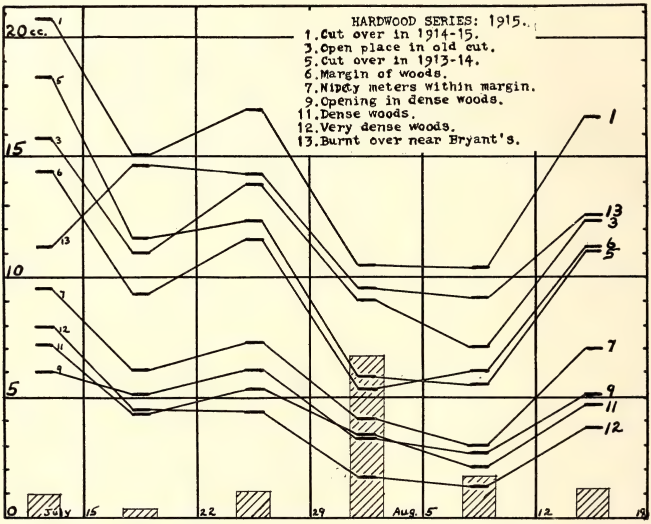

FIG. 2. Diagram showing the daily rate of evaporation in cc. and the precipitation in cm. for the intervals between readings from certain stations in the hardwood series, 1915.

The principal trees in the hardwood or beech-maple association are *Fagus grandifolia*, *Acer saccharum*, *Tsuga canadensis*, *Betula lutea*, and *Tilia americana*. Shrubs occur largely in openings, *Acer pennsylvanicum* being most abundant. A large number of herbaceous species grow near the ground. Among the more frequent of these are *Aralia nudicaulis*, *Maianthemum canadense*, *Trillium grandiflorum*, *Trientalis americana*, *Aster macrophyllus*, *Streptopus longipes*, *Streptopus roseus*, *Medeola virginiana*, *Clintonia borealis*, and *Actaea alba*.

Clearings made in different years, now covered with mixtures of vegetation, furnish series from bare ground up to the hardwood association. Another series leads from bare ground, through aspens, to the pine association; while a third series leads from open water to the *Thuja* association. In wet soil, seedlings of *Thuja* are present under many conditions. On sandy land, healthy seedlings of *Pinus strobus* and *Pinus resinosa* occur under a large number of conditions. Similarly on the better soil are seedlings of *Acer saccharum* and *Fagus grandifolia*. On other portions of the lumbered land such seedlings have become small trees, with every prospect of reproducing the original forest. For the purposes of the present investigation, young seedlings, 15 to 25 cm. in height were chosen, as these are in a most critical stage.

No.	Location.	Cc. in 47-Day Period.
	East Point Series.	
27.	Edge of dune.	618
28.	Dense Thuja Bog.	330
29.	Very dense part of bog.	225
30.	Densest part, no green plants.	155
	Reese's Bog Series.	
31.	Clearing.	587
32.	Layered Thuja in opening.	460
33.	Dense, no green plants.	435
34.	Marginal foss.	391
35.	Thuja seedling in dense part.	345
36.	Bog in Aspens.	335
	Series East of Douglas Lake.	
37.	Thuja seedling in slashed bog.	344
38.	Thuja seedling in slashed bog.	307
	Bryant's Bog Series.	
39.	Edge of Chamaedaphne Assoc.	590
40.	Picea, Pinus and Larix seedlings in Chamaedaphne Assoc.	555

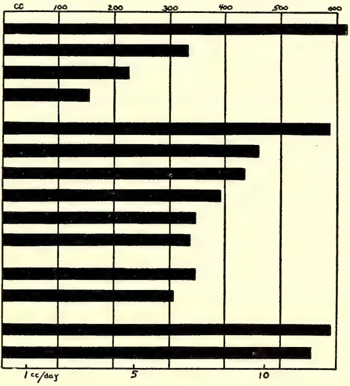

FIG. 3. Diagram showing the total evaporation and the rate per day for 47 days (July 8 to August 24, 1916) from different bog stations.

In 1915 three atmometers were run at the level of *Acer saccharum* seedlings in the dense hardwood forest under three conditions: atmometer No. 11 under ordinary shade (Fig. 6), No. 12 under very heavy shade, and No. 9 in an opening caused by the removal of a large tree. Atmometers No. 6 and No. 7 were run in a large patch of vigorous 1 to 3 year old seedlings near the edge of the forest. The edge was the result of the preceding winter's clean-cut lumbering—therefore exposed to light and wind (Fig. 5). Atmometer No. 6 was placed at the very edge, while No. 7 was run about 90 meters in the forest. Atmometers No. 1 and No. 2 were run alongside of Acer seedlings, growing unprotected in the open sun in an area cleared during the preceding winter (1914–15). In this same area three atmometers were started in 1916. The second year had allowed the brambles to encroach upon the fireweeds—clothing the ground with a dense covering of vegetation. The maple seedlings were likewise one year older and their vigor was positive proof that they were amply and easily meeting conditions. The introduction of cattle into the area in the middle of the summer necessitated the withdrawal of the atmometers. The healthy condition of the seedlings in the fall, however, was evidence that these seedlings could withstand even such an extremely dry summer as that of 1916.

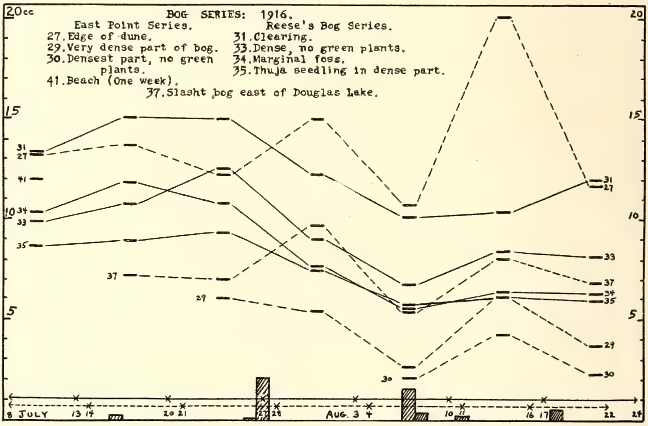

FIG. 4. Diagram showing the daily rate of evaporation in cc. for the intervals between readings from certain bog stations, 1916. The daily precipitation is shown on the same scale in cm.

In 1915, atmometers No. 4 and No. 5 (Fig. 7) were run near maple seedlings in an area cleared in the winter of 1913–14. Weeds and brambles were also present. Atmometer No. 3 was run by maple seedlings in an open place in a thicket-tree growth—long since cut and lightly burnt—into which brambles have entered thickly. Atmometer No. 8, the last of this series, was run on Pine Point in a mixture of hardwood and cedar in which all the large Thujas had been cut out.

A similar series of experiments was run in connection with the establishment of pine plants in the aspen association. Pine seeds are furnished by large trees bordering the lake and scattered sparingly in the main body of the pine land. The ground conditions are various. Open sandy soil may be quite plantless where fire damage has been very severe. The ground is sometimes covered with a dense carpet of moss or sod, which makes seeding ineffective.

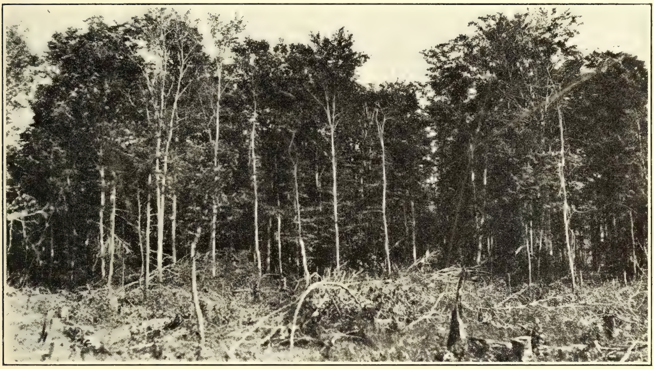

FIG. 5. Margin of hardwoods, the result of clean cut lumbering. July 14, 1916.

Eleven atmometers were set out on a line running back from the lake near the Biological Station under conditions as follows: No. 20 in an open growth of aspen, the ground covered with Pteris; No. 18 near the preceding in a growth of Pteris under the open sky; No. 26 at the foot of a slope in a dense aspen thicket, in which the ground was entirely obscured by the luxuriant growth of Pteris; No. 21 about 20 meters from the preceding at the crest of a slope where the ground flora was predominantly formed by *Gaultheria procumbens* under a fairly open aspen thicket (Fig. 8). Atmometers No. 24 and No. 25 were run in a dense aspen thicket, where Pteris was also luxuriant. This thicket was separated from the uplands by a steep partially cleared slope about 10 meters high. Atmometers No. 17 and No. 19 were run on this slope. On the uplands there were fewer pine seedlings, both because of the distance from seed trees and the greater fire damage. Three atmometers were run in close proximity to small pine seedlings, two of which, No. 22 and No. 23, were under a fairly dense aspen stand, while No. 16 was exposed to the sky.

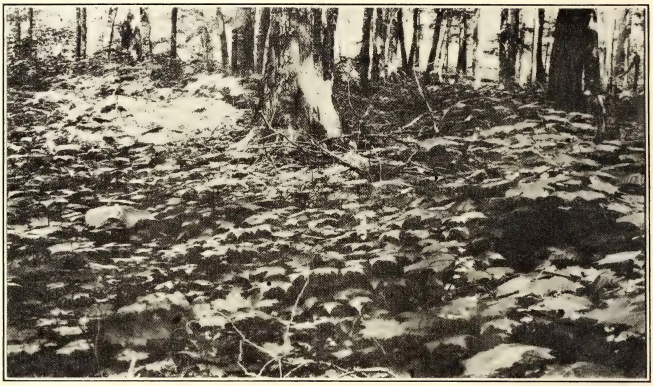

FIG. 6. Floor of a hardwood or beech-maple forest showing atmometer No. 11 in a dense mass of *Acer saccharum* seedlings. Seedlings of *Acer pennsylvanicum* are also present. July 22, 1915.

Until the winter of 1911–12, south of Bryant's hotel, there was a patch of hardwood. East of it was pineland, now vegetated with a very open growth of aspen. A north and south ravine sharply separated these two areas of different vegetation. As the area to the east is in line with the prevailing westerly winds, it has had abundant opportunity to become thoroughly seeded with *Acer saccharum* and other hardwood plants. The hardwood was cut in the winter of 1911–12 and fireswept in May, 1915. To determine whether there was any particular characteristic of evaporation which possibly could have influenced the fact that Acer seedlings were not present in the pineland, although present on the hardwood land, two atmometers were run—No. 13 in the burnt-over hardwood land and No. 15 about 200 meters distant in the pine land.

The evaporation conditions attendant upon the establishment of Thuja seedlings in boggy soil were investigated with 16 atmometers in 1916. Seed trees of Thuja are smaller, less abundant, and more localized in their distribution than pine or maple, which explains why Thuja was not found in some of the smaller bogs. Atmometers No. 39 and No. 40 were run in a small Chamaedaphne bog in which Larix and *Picea mariana* were conspicuous invaders. This bog has been thoroughly fireswept and no Thuja is present. Atmometers No. 41 and No. 42 were started by Thuja seedlings on the beach and at the edge of the beach thicket respectively, but after the first week had to be discontinued. In a small slashed bog along a little stream east of Douglas Lake, atmometers No. 37 and No. 38 were run in moderately open conditions near healthy Thuja seedlings. At East Point there are several bogs in different stages of development. Atmometer No. 27 was run near a Thuja seedling at the edge of the fringing dune, exposed to winds from the lake, No. 28 near Thuja seedlings at the inner edge of the bog, No. 29 in the densest part of the bog in which a Thuja seedling could be found growing, while No. 30 was run, in August, in the deepest and darkest spot which could be found. Thuja seeds but no Thuja seedlings were present. Atmometer No. 36 was run in a small bog in the aspens south of the Biological Station. Larix, Thuja and Picea were present, but fire had seriously damaged the vegetation.

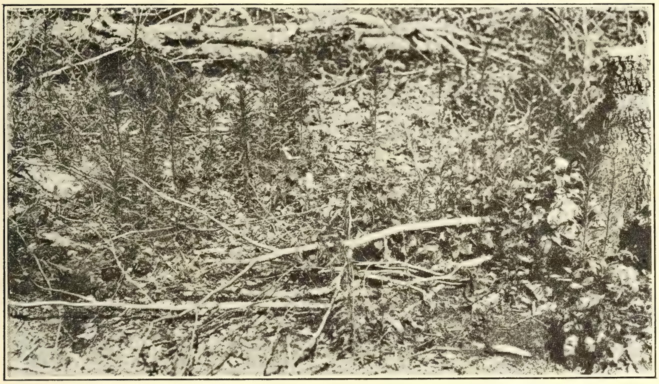

FIG. 7. A view in a hardwood area cut over in 1913–14, showing atmometer No. 5. The conspicuous weed is *Erigeron canadensis*. July 22, 1915.

Reese's bog, the largest bog in the vicinity of the Biological Station, is a well-developed Thuja bog. Atmometer No. 34 was run near a Thuja seedling in the marginal foss at the foot of a hill, where the soil was very wet. Although exposed to the sun, the opportunity for free circulation was poor. Atmometer No. 35 was near a Thuja seedling in a dense thicket of 10–20-foot saplings in very wet soil—likewise hemmed in from the wind. Atmometers No. 32 and No. 33 were in Thuja on slightly higher ground where the soil was dry at the surface and the circulation good—No. 32 in a slight opening in which a layered sprout was healthily growing and No. 33 in a very dense thicket of small trees under which was no green ground vegetation (Fig. 9). Ungerminated composite and Thuja seeds were found in the layer of dead Thuja leaves. Atmometer No. 31 was run by a Thuja seedling in a good-sized clearing where the seedlings were exposed to full sunlight.

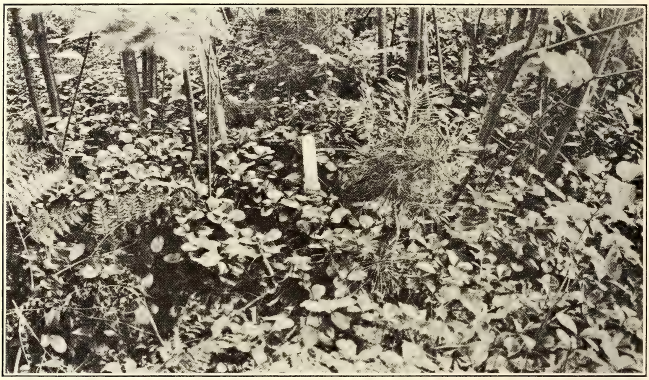

FIG. 8. View showing atmometer No. 21 near a pine seedling at the crest of a hill in an open aspen growth. The ground is carpeted with *Gaultheria procumbens*. August 9, 1915.

Atmometer No. 14 represents the evaporation conditions of the bare ground near the lake in the immediate vicinity of the laboratory, in 1915.

In each experiment, unless otherwise noted, the atmometer was run in immediate proximity to a young healthy seedling of maple, pine, or white cedar and represents the conditions successfully met by those seedlings. Where virgin hardwood forest is cleared during a winter, the vegetation in the following spring consists of such forest species as can withstand the new conditions. This includes the seedlings of *Acer saccharum*. Weeds appear later in the season, but not in great abundance during the first year. During this time maple seedlings have little or no protection from the full sun, yet large numbers of them survive. Is a downward change in evaporation a necessary prerequisite to succession or is the evaporation changed as a result of succession? If the former is the case, since *Acer saccharum* seedlings are normal to the floor of the climax vegetation where the rate of evaporation is very low, it might be logical to suppose that maple seedlings will not be found except where the rate of evaporation is much less than that over bare ground. If the latter is the case, maple seedlings will be found growing wherever the soil is suitable, regardless of the rate of evaporation of the habitat and regardless of any change that their development may subsequently have upon the habitat.

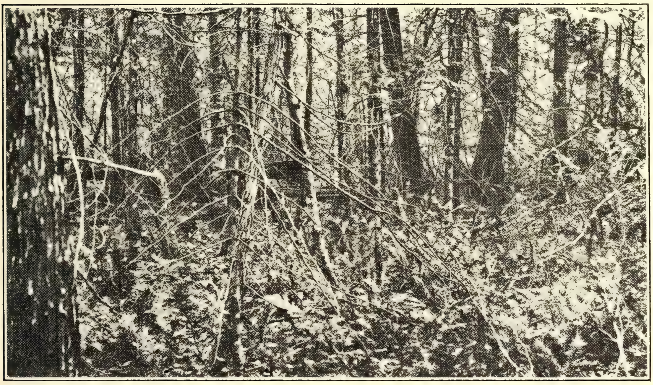

FIG. 9. View in a Thuja bog, showing atmometer No. 33 in the center of the background where the shade is so dense that no green ground vegetation is present. August 12, 1916.

The results and their interpretation follow: Taking up the hardwood series first, the following results were obtained. In the area cut over during the winter of 1914–15, where sufficient time had not yet elapsed for weeds to invade and change the evaporating conditions of the ground layer, the rate of evaporation was 590 and 561 cc. for 40 days in the middle of the summer of 1915. This rate was 3.37 times as great as that from the floor of the normally dense hardwood forest in this region. In the area cut during the winter of 1913–14, where weeds and brambles had entered in quantity, the evaporation rates were 416 and 425 cc. from two stations. A relative slowing up of the rate of evaporation even during the season was plainly evident in atmometer No. 4, as the development of weeds during the course of the season came to protect the instrument and the Acer seedling to a greater and greater degree. In fact this protection from weeds was sufficient to cause a lower rate of evaporation than was obtained from atmometer No. 3 run in an open weedless spot in an area where hardwoods had made considerable progress in revegetating a former cut. There, the rate was 453 cc. during the same length of time. At the edge of the woods, where atmometer No. 6 was stationed in a luxuriant growth of *Acer saccharum* seedlings, a rate of 378 cc. was obtained for the period of experimentation. Ninety meters in from the margin, the rate had decreased to 240 cc. Within the woods the rate was 175 cc. in a spot of average density, 147 cc. in a very dense situation, and 187 cc. in a small opening in the dense forest. These results show a wide range of conditions from bare ground without shade—the severest conditions maple seedlings could be called upon to withstand—to the mature forest with its dense shade. Seedlings in the open received sunlight. Under more advanced conditions in the vicinity it was seen that such seedlings were developing into trees, while the vast majority of the multitudes of seedlings in the dense forest did not persist for more than a year or two, unless they were in openings.

This is a clear case in favor of the contention that the seedlings of the dominant species of certain associations become established irrespective of the evaporation conditions—in fact, with the additional advantages accruing from an increased amount of sunlight, seedlings of mesophytic species thrive better under more xerophytic conditions than that which the mature forest furnishes.

In the presence of sunlight, Thuja seedlings readily develop in either sandy or boggy soil, having a sufficient supply of water, under the entire range of evaporation conditions present in the region. Thuja seedlings commence development on the open beach, but are destroyed by ice action. On the low fringing dune, where the evaporation was 618 cc. from atmometer No. 27, Thuja seedlings were more frequent.

At Bryant's bog, where conditions were intermediate between the sand dune and a normal cedar bog, atmometers No. 39 and No. 40 gave 590 and 555 cc. respectively for the season of 1916. As previously noted, this bog has been repeatedly devastated by fire and there are no Thuja seed trees in the immediate vicinity. The absence of Thuja, therefore, can not be attributed to the conditions of evaporation.

In certain of the East Point bogs, conditions pre-eminently suitable for the development of Thuja prevail. Although Thuja seedlings are found under a wide range of evaporation conditions, there are places in the bog where it is too dark for them to grow. Darkness is here attended by low evaporation. With an increase in light, evaporation is increased. Since a certain amount of light is necessary for the development of the Thuja seedling, low evaporation is not in itself a sufficient reason for the absence of Thuja seedlings. Darkness results from the dense canopy formed by the trees, but even in the darkest places Thuja seeds may be found. The evaporation from such a spot where no Thuja seedlings were present was 155 cc. for the season of 1916. In a small opening nearby, where Thuja seedlings were actively growing, the evaporation was 225 cc. The increased rate of evaporation in itself could hardly be held responsible for the presence of seedlings in one case and not in the other. The development of seedlings in openings tends to restore a dense canopy and thus to lower the evaporation from the chamaephytic layer. When a clearing of considerable size is made, the evaporation is increased to a much greater extent, as in the case of atmometer No. 3, in Reese's clearing with an evaporation of 587 cc. Many Thuja seedlings were present.

Reese's bog occupies a low rolling site at the head of Burt Lake. A road and several trails improve its circulation. A comparison of atmometers No. 33 and No. 35 brings out the effect of circulation. Atmometer No. 33 on the ground beneath a canopy of Thuja so dense as to prevent ground vegetation, gave 435 cc., a higher rate than 345 cc. from No. 35 in the crown of a small Thuja seedling in an opening nearby. In the latter case, the development of edge conditions in the foliage of the trees around the opening greatly checked the circulation. Likewise atmometer No. 34 by a small Thuja seedling in the marginal foss at the foot of a high ridge, where air drainage was poor, gave 391 cc., a lower result than the 435 cc. from No. 33, which was further in the bog, but free from the influence of ground vegetation owing to the dense canopy of Thuja saplings.

Atmometer No. 36, run in a small relic bog in the aspens north of Reese's bog gave 335 cc. and atmometers No. 37 and No. 38, run in a slashed bog to the east of Douglas Lake, gave 344 and 307 cc. respectively. In each case Thuja seedlings were developing at a rapid rate. Based upon one week's record, the evaporation near a Thuja seedling on the beach for the season of 1916 would have been 562 cc. and 487 cc. at the edge of the beach thicket.

In the pine series, investigated during 1915, the evaporation varied from 347 cc. in an open spot in an aspen grove, through 310 and 321 cc. on an exposed hillside, 197, 198, 196, and 187 cc. in the ordinary aspen association to 178 cc. at the foot of a slope in the densest part of the aspens. In each case the results express the conditions withstood by one to three-year-old pine seedlings of which there were large numbers throughout the aspens. Pine seedlings easily withstand as wide a range of conditions as the region presents. In no case therefore could it be said that evaporation conditions were the determining factor in their ecesis. The presence of all ages and sizes of pine trees is excellent evidence of how well the pine is developing and in consequence the succession is progressing. Aspen seedlings are abundant in the open sandy ground. As they develop, the increasing shade and the checking of the wind are instrumental in causing a decrease of evaporation from the chamaephytic layer, for example, atmometers No. 18 and No. 24, with rates of 315 and 196 cc. respectively.

The two atmometers run during 1915 in connection with the area south of Bryant's, the one in pine land and the other in hardwood land —each of which was very openly vegetated—gave the following results: The evaporation from the immediate vicinity of a pine seedling in pine land was 513 cc. Atmometer No. 13, run in hardwood land devastated by fire, one and one half months previous, gave a rate of 473 cc. for the same period. The fact that the evaporation rate was 473 cc. in the hardwood land, where maple seedlings were present, and 513 cc. in the pine land, where pine seedlings were present, whereas maple seedlings developed successfully under the highest rate (590 cc.)

obtained in the region, means that the evaporation from the chamaephytic layer is not the fundamental factor in the ecesis of such seedlings.

The fact that the rate of evaporation from the chamaephytic layer is decreased in the development of mesophytism has been demonstrated by many investigators: Transeau (5) at Cold Spring Harbor, Gleason and Gates (1) in Central Illinois, Fuller (2) near Chicago, Weaver (3) in southeastern Washington and adjacent Idaho, and the present investigation in northern Michigan all strongly bring out the same conclusion. If there is a causal relationship between evaporation from the chamaephytic layer and succession, which I believe no one disputes, either the decreased evaporation causes plant succession or plant succession causes a decrease in evaporation. Dr. Gleason and I (1) made the latter interpretation. Fuller (2) says: "the decreased rate of evaporation caused by the heavier vegetation is the direct cause of succession between different associations." The data of the present investigation indicate that evaporation is changed in the course of succession and not preceding it.

In the succession towards mesophytism a conspicuous feature is the fact that the seedlings of the dominant species of a genetically higher association commence their development under the conditions furnished by the existing association. Instead of a change of evaporation preceding the development of a different vegetation, that which is controlling and changing the rate of evaporation from the chamaephytic layer is the invading dominant species which have successfully withstood the conditions imposed upon them by the existing association. It is quite obvious that they can not change nor control conditions before they are present.

An increase in density of an association, itself, likewise causes a decrease in the rate of evaporation from the chamaephytic layer. Except a change of dominant species obtain, however, succession has not taken place. Variations in evaporation from typical stations of a given association in a given area are not likely to be as great as the difference obtained between two genetically related associations. If one should add to Fuller's statement, previously quoted, to have it read: "The evaporation thus controlled and changed is one of the principal factors in permitting the development of a different *lower story* vegetation," its validity could be readily appreciated for those secondary species whose physiological limitations precluded their development in the lower genetic association. The fundamental thing in succession is the replacement of the dominant species of the existing association by those of the invading association. Changes in the flora of the ground layer are secondary events.

With these facts in mind, one can not dodge the issue that, in a given local area, invasion takes place under the existing conditions. With the development of the invading species the evaporation conditions of the ground layer are changed, which is usually accompanied by a change in the *ground* flora. In other words, a change of evaporation conditions of the ground layer is the result and not a fundamental cause of succession.

SUMMARY

1. Experimentation was carried on in the vicinity of Douglas Lake, Michigan, during the summers of 1915 and 1916, with 42 standard Livingston atmometers. The usual methods of experimentation and calculation of data were employed.

2. As the investigation was carried on in a small area, the influence of edaphic factors was not obscured by the action of broad climatic factors.

3. Invasion, which is the initial stage of succession, must take place under the conditions already existing.

4. The change of conditions coincident with mesophytic succession brings about a decrease in the rate of evaporation in the ground or chamaephytic layer.

5. In a given area, the differences in the amount of evaporation under which seedlings develop are largely due to the surrounding vegetation, which by its size and density controls the evaporation beneath it.

6. The complete range of evaporation conditions present in this region, namely, from bare ground to the mature forest, is completely within the physiological limits of the seedlings of *Acer saccharum*, *Pinus strobus*, *Pinus resinosa*, and *Thuja occidentalis*. Given suitable soil conditions, maple seedlings will develop under evaporation conditions at least 337 percent more xerophytic than the normal hardwood forest, or 400 percent more xerophytic than the very dense forest.

7. Within their soil requirements and in the presence of light, the establishment of the pine, beech-maple and Thuja bog associations—three of the most important tree associations in northeastern North America—is independent of any particular conditions of evaporation. Consequently a decrease in evaporation is not a prere-

quisite to succession. A change in dominant species in an area is fundamental to succession.

8. The change in the rate of evaporation from the chamaephytic layer is produced by the development in density of the invading vegetation. Being coincident with and not antecedent to it, the change in evaporation is a result and not a cause of succession.

9. While it is necessary for certain species to develop under existing conditions to bring about succession, other species, of narrower physiological limitations, can not develop until conditions are brought within their range. Such species are secondary species, unable to cause succession.

10. Even though evaporation conditions are within suitable limits, succession will not take place unless the disseminuls of the dominant species of a higher genetic association arrive and develop.

11. The average evaporation from the chamaephytic layer of the average aspen association for 40 days during the summer of 1915, at Douglas Lake, Michigan, was 4.9 cc. per day; for the normal density of the beech-maple forest, 4.4 cc. per day; while the highest average rate for the season obtained from open ground was 14.7 cc. per day. For a single week the highest rate was 21.6 cc. per day.

For 47 days during the summer of 1916, the average evaporation from the chamaephytic layer of a densely developed Thuja bog was 4.8 cc. per day. A rate of 26.6 cc. per day was recorded from an atmometer in open ground at the crest of the low bluff a short distance from the laboratory.

List of Plants Mentioned, with Authorities

(Using "Gray's Manual," 7th Edition. Where different, the names used in Britton and Brown "Illustrated Flora," 2d edition, are given in parentheses.)

Acer pennsylvanicum L.
Acer rubrum L.
Acer saccharum Marsh.
Actaea alba (L.) Mill.
Agrostis alba L.
Agrostis hiemalis (Walt.) B.S.P.
Aralia nudicaulis L.
Aster laevis L.
Aster macrophyllus L.
Betula alba papyrifera (Marsh.) Spach. (*B. papyrifera* Marsh.).
Betula lutea Michx. f.
Chamaedaphne calyculata (L.) Moench.
Clintonia borealis (Ait.) Raf.
Convolvulus spithamaeus L.
Cornus canadensis L. (*Chamaepericlymenum canadense* Asch. & Graebn.).
Danthonia spicata (L.) Beauv.
Diervilla lonicera Mill. (*D. diervilla* MacM.).
Epilobium angustifolium L. (*Chamaenerion angustifolium* Scop.).

Erigeron canadensis L. (*Leptilon canadense* Britton).
Erigeron ramosus (Walt.) B.S.P.
Fagus grandifolia Ehrh.
Fragaria virginiana Duchesne.
Gaultheria procumbens L.
Habenaria obtusata (Pursh) Richards. (*Lysiella obtusata* Richards.).
Hieracium scabrum Michx.
Hieracium venosum L.
Larix laricina (DuRoi) Koch.
Ledum groenlandicum Oeder.
Lepidium virginicum L.
Maianthemum canadense Desf. (*Unifolium canadense* Greene).
Medeola virginiana L.
Melampyrum lineare Lam.
Mitella nuda L.
Moneses uniflora (L.) A. Gray.
Oryzopsis asperifolia Michx.
Panicum xanthophysum A. Gray.
Picea mariana (Mill.) B.S.P.
Pinus resinosa Ait.
Pinus strobus L.
Pirola asarifolia incarnata (Fisch.) Fernald (variety not given in Britton and Brown).
Poa pratensis L.
Populus grandidentata Michx.
Populus tremuloides Michx.
Prunus pennsylvanica L.f.
Pteris aquilina L. (*Pteridium aquilinum* Kuhn).
Quercus rubra L.
Rhus glabra L.
Rubus allegheniensis Porter.
Rubus idaeus aculeatissimus (C.A.Mey.) Regel & Tiling (*Rubus strigosus* Michx.).
Rubus triflorus Richards.
Rumex acetosella L.
Salix rostrata Richards. (*Salix bebbiana* Sarg.).
Smilicina stellata (L.) Desf. (*Vagnera stellata* Morong).
Solidago canadensis L.
Streptopus amplexifolius (L.) DC.
Streptopus longipes Fernald. (Included with the following species in Britton and Brown).
Streptopus roseus Michx.
Thuja occidentalis L.
Tilia americana L.
Trientalis americana (Pers.) Pursh.
Trillium grandiflorum (Michx.) Salisb.
Tsuga canadensis (L.) Carr.
Vaccinium oxycoccus L. (*Oxycoccus oxycoccus* MacM.).
Vaccinium pennsylvanicum Lam. (*Vaccinium angustifolium* Ait.)
Viburnum acerifolium L.

Carthage College,
Carthage, Illinois.

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CONTENTS

The relation of some rusts to the physiology of their hosts. E. B. MAINS 179

The development of some species of Agarics . . . . A. W. BLIZZARD 221

The origin and development of the galls produced by two cedar rust fungi. J. L. WEIMER 241

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AMERICAN
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THE RELATION OF SOME RUSTS TO THE PHYSIOLOGY OF THEIR HOSTS[1]

E. B. MAINS

I. INTRODUCTION

The relation of the rusts to their hosts has long occupied the attention of many workers, not only because of their economic importance, but more especially by reason of the extremely interesting biological problems which they offer. Not only have the rusts afforded a wide field for the study of the questions of immunity, susceptibility, physiological varieties, heteroecism, etc., but they, together with a few other groups such as the Peronosporales and Erysibaceae, make up part of the group of fungi which de Bary has called obligate parasites. This group of fungi is characterized by the requirement of a living host as the source of food supply. Saprophytes, on the other hand, obtain their food from dead organic material. Between the two classes are the intergrading facultative parasites and facultative saprophytes, determined by the degree a fungus is independent or dependent upon a living host. The saprophytic and facultative parasitic fungi have long been studied with attention to their food relations, but most of the work upon the obligate parasites has been confined to other lines, since the parasitic condition itself puts great difficulties in the way of an investigation of the nutrition of the fungus.

One must not overlook the fact that there are two conditions in obligate parasitism. In the first, we have the problems concerned with immunity and susceptibility, a condition which is common to all parasites whether obligative or facultative as well as to the facultative saprophytes. The other condition is that which goes to produce the

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[1] Paper No. 156 from the Botanical Department of the University of Michigan.

obligative relation and make it seemingly impossible for the fungus to grow elsewhere than upon its host or hosts. Much work has been done and a number of theories developed with reference to the first condition; but concerning the second only a little work has been attempted, and but few theories advanced. Some authors in an endeavor to explain such parasitism have expressed the idea that the solution of the problem might be sought in the requirement of the fungus for some special nutrient which only its own particular host would be able to supply. What this nutrient might be, if such is the case, would be of extreme importance. Failing to determine this, it would be of not much less importance that some idea of its nature be obtained. Since the obligate parasites are distinguished by the absolute need of a living host for their food supply, it is from the host that the evidence for the solution of such a problem must be sought, and it is through the control of the various physiological activities of the host that one may hope to do this. It was to this end that this work was undertaken with the object of obtaining more data regarding the factors which control the obligate condition and determining, if possible, the substances or class of substances which are necessary for parasitism of this kind.

The work was carried on in the Cryptogamic Laboratory of the University of Michigan during the years 1914, 1915, and 1916 at the suggestion and under the direction of Dr. C. H. Kauffman to whom I am under deep obligations for many helpful suggestions and stimulating criticism.

II. HISTORICAL

The early history of the parasitism of the rusts has been well summarized by de Bary (1853), who was the first to study the rusts and smuts with scientific accuracy. According to de Bary, early naturalists such as Pliny, Theophrastus, Malpighi, Duhamel, Tillet, Tessier, and Plenk considered rusts not as the cause but as the result of a diseased condition brought about by atmospheric conditions. In the course of time, they were looked upon as foreign material which was partly the cause and partly the result of the disease. Later, the rusts were recognized as fungi by Linnaeus and Persoon, but they were still considered as the product of a diseased condition due to an injury such as the sting of an insect, etc. Unger (1834) believed that the rusts were produced by disarrangements in the respiratory organs of the plant due to which sap exuded into the intercellular spaces and there coagulated, thus forming the rust. The next great step in the direction of a true understanding of the nature of the rusts was the recognition that they were the cause of the disease. The believers in this theory first concerned themselves with the study of the rusts as fungi and their entrance into their hosts. Léveillé (1839) showed that the rusts did not differ from saprophytic fungi in their development of mycelium and fruiting bodies except that they were within the living host. Prevost, according to the Tulasne brothers (1847), first observed the germination of rust spores. The Tulasne brothers and de Bary (1853, 1863) showed that the germ-tubes of the rusts enter through the stomata of their hosts and in some cases (the germ tubes of basidiospores) through the cell wall.

It was de Bary (1853), however, who finally definitely established that the rusts were parasites and that they were the cause and not the result of the disease. He concluded that the "Brandpilze," in which group he included both smuts and rusts, are to be considered as parasitic growths, since they arise from spores whose germ-tubes penetrate the host, develop a mycelium within the host's tissue, form spores, and finally break through the epidermis and infect other plants. De Bary in Die Brandpilze (1853, p. 109) defined a parasite as, "solche Pflanzen oder Thiere, welche auf lebenden Geschöpfen existiren, und ohne diese nicht bestehen können, welche durch den Reiz, den sie verursachen, durch die Nahrung, die sie dem Wohnorganismus entziehen, Störungen in dessen Organfunctionen hervorrufen; diese schwinden, sobald der Parasit entfernt oder getödtet wird." In view of this definition, de Bary's work on the "Brandpilze" was hardly sufficient to establish the rusts as parasites, since he did not show th they could not exist outside of living organisms.

A rather exhaustive search of the literature of this period does not reveal that any attempts were made to grow the rusts saprophytically. The general opinion which is now held appears to have arisen from the earlier idea that the rusts were diseased products of the host, first non-living and finally living products. In part this assumption of the obligate character of the rusts is due to the fact that they, unlike the facultative parasites, are never found in nature growing on other than living plants.

Among the later workers upon the obligate parasitism of the rusts is Brefeld (1883, 1908), who believes that the growing of rusts saprophytically is merely a matter of technique. He was able to obtain secondary and tertiary spores from the basidiospores of some rusts, but further development was prevented by contaminations present in his cultures. Carleton (1903) used different media and a substratum as nearly like a wheat leaf as possible and obtained only a little difference in the length of the germ-tubes. Carleton, however, does not give an account of his methods. Ray (1901, 1903) has claimed to have cultivated a number of rusts upon decoctions of the host and on sterilized carrot and reports that in one case teleutospores were formed from the mycelium which was produced. Ray, however, gives only a very imperfect account of his methods. This coupled with the fact that he does not give either the species of rust or the kind of spores used subjects his results to criticism.

The germination of rust spores has received considerable attention especially from later workers. Plowright (1889), Eriksson and Henning (1894), Ward (1902*b*, 1903), Melhus (1912), Johnson (1912) and myself (1915) have found that temperatures between 10° and 30° C. are necessary for good germination and that the optimum temperature is between 12 and 18° C. Fromme (1913) has shown that a saturated atmosphere is necessary for abundant infection.

The factors controlling the inoculation of the host after the germination of the rust's spore have been principally investigated by Ward and his students. Miss Gibson (Ward, 1905) found that the germ-tubes of a number of rusts are able to penetrate into the intercellular spaces of plants other than their host without infecting. Furthermore Ward (1905) noticed this in the case of immune varieties of plants. He (1902*b*) also observed that the germ-tubes of *Puccinia dispersa* had a tendency to be negatively heliotropic and suggested that this may be a factor aiding in inoculation. Robinson (1914) in the case of *Puccinia Malvacearum* and Fromme (1915) and myself (1915) in the case of *Puccinia coronata* have shown a similar reaction of the germ-tubes. Balls (1905) believes that inoculation is brought about by a hydrotropic stimulus which causes the germ-tube to enter the stoma of its host.

The relation of the rusts to their host after infection has occupied the attention of a number of workers. De Bary (1887) and Jost (1907) have expressed the opinion that the predisposition of certain hosts for certain parasites is to be sought in the nature of the food which that host offers to them. De Bary (1887) and Tubeuf (1897) both have remarked that the rusts appear to adapt themselves to their host cells, causing but little injury, at least up to the time of spore formation. Tubeuf, in the case of hypertrophies caused by rusts, thought that there appeared to be "a closer symbiotic relation between the fungus and its host branch than between the host branch and its main branch." He also noticed that in the case of some rusts the infected portion of the leaf remained alive after the death of the surrounding tissues and he looked upon this as a condition resembling that of some lichens. Ward (1890, 1902*c*) suggested that the relation between *Puccinia dispersa* and its host tends towards symbiosis and that the rust is not destroying the protoplasm of the host, but is robbing the host of its food supplies. Ward (1905) found that when the rust does attack the host so vigorously that the protoplasm is killed it brings about its own death and immunity for the host is produced. This condition, he discovered, can be duplicated by starving the host and by so doing starving the rust. His earlier work (1902*a*, 1902*c*) had already pointed towards this conclusion since he found that immunity did not depend upon anatomical features such as number and size of the stomata, hairiness, etc., and that mineral starvation, although it did not produce immunity, reduced the number of spores which were produced.

The relation of the rusts to the carbohydrate supply has been noticed by a number of workers. Halsted (1898) found in the case of *Puccinia Podophylli* that there is a collection of starch in infected regions of the leaf. The centers of such areas however contained much less starch than the margins. Robinson (1913) noticed much less starch in those areas of the leaf which are infected with *Puccinia Malvacearum*. McAlpine (1906) quotes the work of F. T. Shutt, who found that the grain and straw of rusted wheat contained more protein and less carbohydrates than the rust-free plants. Tischler (1912), working with *Uromyces Pisi*, discovered that the portion of the host containing the most mycelium of the rust also contained the greatest amount of sugar.

The effect of environmental factors such as soil, moisture, temperature, and light upon the relation of the rusts to their hosts has been studied by a number of workers. McAlpine (1906) noticed that nitrogenous manures retarded the ripening of grain, while phosphate of lime brought about early maturity and enabled the grain to escape the rust to some extent. Sheldon (1905) has reported that soils favorable to the host are also favorable to the rust of carnations (*Uromyces Caryophyllinus*).

Considerable difference of opinion has arisen concerning the effect of moisture on the development of the rusts. De Bary (1863) found that vegetative development of *Uromyces appendiculatus* and its production of spores was greatly increased by a humid atmosphere. McAlpine (1906) reported that drainage increased the yields of wheat, but did not decrease the rust. He also considered that irrigation late in the season tended to make the grain soft and brought on the rust. Stone and Smith (1899) and Blaringhem (1912) claimed that the rusts were favored by dryness. Sirrine (1900) and Buchet (1913), on the other hand, claimed that the rusts were favored by wet soils. Smith (1904) considered that a dry atmosphere retarded the development of the rust within the host while a dry soil favored development. Fromme (1913) found that for *Puccinia coronata* after infection has taken place moisture appears to have no effect upon the length of the incubation period.

The effect of temperature upon the development of rusts in their hosts has been but little studied. Sheldon (1902) found that the incubation period of *Puccinia Asparagi* was longer at an average temperature of 69° F. during the winter months than at an average temperature of 76° F. during the summer. It is likely that these results were in part due to the difference in the amount of light present in the two seasons. Fromme (1913) discovered that for *Puccinia coronata* a temperature between 20° and 30° C. brings about a shorter incubation period than a temperature of 14° to 21° C.

Fromme (1913) appears to be the only one who has definitely investigated the effect of light upon the development of the rusts. He found that when oats inoculated with *Puccinia coronata* were placed in darkness for a few days and then returned to the light, the incubation period was lengthened approximately by the time that the oats were in the dark. Fromme states that this may mean a dependence of the rust upon the transition products of photosynthesis and that this may explain the obligate parasitism of the rusts.

III. EXPERIMENTAL WORK

MATERIAL

Two rusts were employed, *Puccinia coronata* Cda. and *Puccinia Sorghi* Schw. *Puccinia Sorghi* was used in all of the experiments since its host maize (*Zea Mays* L.) was easier to work with. *Puccinia* *coronata* was employed wherever oats (*Avena sativa* L.) could be used advantageously, and whenever time allowed. These two rusts were kept going on their host plants and thus stock material was always on hand. The method used was to make a spore suspension of the uredospores and spray them on the host by means of an atomizer. The pots were then covered with a belljar and placed where the temperature ranged between 14° and 25° C., a temperature of 20° C. being used whenever obtainable. The belljars were removed after 24 hours. When maize was used it was found to be advantageous to draw the leaves gently between the fingers before inoculating as the leaves are covered with a wax-like substance which causes the spore suspension to roll off without wetting them. In some experiments, definite areas were inoculated by placing spores on them with a spear-pointed needle after the plant had been atomized. During the winter, the cultures were kept in a greenhouse, where *Puccinia coronata* required renewal about every three to five weeks and *Puccinia Sorghi* about every two months. This was done by inoculating freshly grown plants. During the summer, the stock cultures were kept in a garden in the open, where the rusts propagated themselves.

Development of the Rusts

Puccinia coronata

The first signs of infection show in from five to seven days after inoculation, when light green areas are formed on the leaves. About seven to eleven days after inoculation, pustules appear in these areas as small, yellowish swellings, which soon break through the epidermis liberating the mass of uredospores. Teleutospores develop in about twenty-nine to thirty-six days after inoculation, when uredospore production has about ceased and the leaf is slowly dying and drying up. They show as blackish zones usually at the margins of the infected areas and their first appearance occurs towards the apex of the leaf, which is also the part of the leaf which first begins to die.

Puccinia Sorghi

The first signs of infection on maize are also light-colored areas on the leaves. These appear in about six to seven days after inoculation and pustules develop soon afterward, in usually seven to ten days.

The pustules make their appearance first on the lower side of the leaf and are more abundant and larger there, often becoming confluent.

The development of the rust within the host can be followed by sectioning day by day after inoculation. The method employed, which gave very good results, consisted in sectioning the infected leaf between pith. Very thin sections can be obtained in this way, if the leaf is cut up and a number of thicknesses of the leaf are placed between pieces of pith. The sections obtained in this way were mounted in Stevens's chloral hydrate and iodine (Stevens, 1911). The sections are cleared by this mixture so that the hyphae of the rust stand out, showing clearly the haustoria in the cells of the host. Chlorophyll, which in untreated sections interferes with the determination of the mycelium, is thus bleached out, and any starch which may be present can be recognized. At the end of the third day, mycelium was already found to be well developed. At this time, the amount of starch, which was normally present in the parenchyma sheaths of the vascular bundles, was only slight, or it was entirely absent. By the fourth day, the mycelium had formed dense masses in the intercellular spaces of the mesophyll of the leaf. None was found sending haustoria into the epidermal cells, nor was any mycelium found in the vascular bundle and its parenchyma sheath. The cells of the parenchyma sheaths in the infected areas did not show at this time so large a quantity of starch as those in the non-infected areas. By the fifth day, the epidermal cells were invaded by haustoria from neighboring hyphae, and the amount of starch was noticeably less in the parenchyma sheaths of the infected areas. About the sixth day, pustules appeared. These were formed from masses of mycelium just below the epidermis. No mycelium was found in the vascular bundles or their parenchyma sheaths at this time. Some starch was found in the parenchyma sheaths of the infected areas, but these did not stain so darkly with iodine as those of the non-infected areas.

The development of the rust progresses by a continued spread of its mycelium and the production of more pustules. The infected areas are however always limited in extent, varying from a millimeter to about a centimeter in diameter. Often, in the case of heavy infection, whole leaves may become covered with pustules due to the union of many infected areas. Mycelium is found throughout the intercellular spaces of the infected areas, where it sends its hyphae into the neighboring cells, forming the branched, finger-like haustoria, which have been well illustrated by Evans (1907). The vascular bundles are apparently never invaded by the mycelium and the parenchyma sheaths which surround them only occasionally have haustoria in their cells.

When limited areas of the leaf are infected, an interesting phenomenon takes place. After the tissue has been infected for some time—in some cases in so short a time as nine days—the ends of the leaf beyond the infected areas begin to die and the regions immediately surrounding become yellowish, while the infected areas retain the green color of healthy tissue. The infected areas, of which there may be two or three on the same leaf, often become surrounded by dead tissue, except perhaps for the midrib itself. The infected areas themselves still retain their original green color, and sections show that the cells of these regions have all the appearance of normal cells, except for the presence of haustoria within them. They are turgid and filled with green chloroplasts. The neighboring tissue, on the other hand, is brown and the cells are shrivelled up and dead.

Effect of Temperature upon Development

Puccinia coronata

Experiments 1 and 2.—Two experiments were carried out to discover the effect of temperature upon the development of *Puccinia coronata* within its host. In each, six pots of oats were used. These were inoculated by spraying with a spore suspension of the uredospores, and, after being left for 24 hours under belljars, four pots of each set were removed to a well-lighted room where the temperature averaged 15° and 13° C. respectively for the two experiments. Two from each set were kept in a similar room where the temperature averaged 20° C. The incubation period of the rust at 20° C. in both experiments was 9 days. The incubation period of the rust at 15° C. in the first experiment was 13 to 15 days, and the incubation period for the rust at 13° C. in the second experiment was 15 days.

The results of these two experiments thus indicate that low temperatures retard the development of the rust in its host.

Puccinia Sorghi

Experiment 3.—This experiment was carried out with *Puccinia Sorghi* on corn in the same manner as were the two preceding experiments. Three pots of corn were kept at a temperature averaging 20° C. and three at a temperature averaging 13° C. The incubation period of the rust at 20° C. was 7 days while that at 13° C. had an incubation period of 13 days, showing that low temperatures retard the development of *Puccinia Sorghi* in its host.

Experiment 4.—Six pots of corn were inoculated with uredospores of *Puccinia Sorghi* and kept under a belljar at a temperature of 18° C. for 12 hours. Two of the pots were placed under belljars in an Eberbach electric incubator at 40° C. The outer door of the incubator was left open, allowing light to enter, and the incubator was placed in an east window. In a similar manner two pots were kept in an incubator at 30° C. Even with the outer doors open these incubators maintained a temperature varying only a few degrees. The other two pots of corn were placed under belljars in a box about the size of the incubators with the open side facing the window. These two pots were at room temperature, which according to a thermograph averaged 18° C. The belljars were removed once a day from all the plants in order to renew the oxygen supply.

At the end of the fourth day, the two pots of corn at 40° C. were dead. Pustules appeared on the plants at 18° C. in seven days. The only sign of rust on those at 30° C. at this time was the greenish spots mentioned before as remaining in infected areas when the rest of the leaf is yellowing. Sections through these areas showed a mycelium which was only sparingly developed. At the end of fourteen days, no pustules had formed on the plants at 30° C. At this time, most of the leaves were dead, only the upper still retaining a green appearance.

These results show that a temperature of 30° C. or higher prevents the development of *Puccinia Sorghi* in its host.

Effect of Humidity upon Development

The work, as far as carried out, was done with *Puccinia Sorghi.*

Experiment 5.—Fourteen plants of maize were inoculated with the uredospores of *Puccinia Sorghi* and kept under belljars at 18° C. for twelve hours. Four plants were then placed in a south window under belljars, by which means they were kept in a nearly saturated atmosphere. The remaining ten plants were placed without belljars on the table beside the other four. The earth in the pots of five of these ten was kept saturated with water. The other five were watered just enough to prevent the plants from wilting. The humidity of the room varied between 20 and 36 percent and the temperature averaged 24° C. At the end of nine days after inoculation the number of pustules on the plants was counted.

The plants in dry air and moist soil averaged 51 pustules per plant. These pustules, however, were small and not as well developed as in the others. Plants in a dry atmosphere and wet soil averaged 151 pustules per plant. These pustules were large and well filled with spores. Plants in a saturated atmospheric and wet soil averaged 371 pustules per plant. These pustules were large and difficult to count as many of them had become confluent.

At the end of 25 days, the infected leaves of plants in dry air and moist soil were all dead and dried up, and the new leaves were free from the rust. The plants in dry air and wet soil had a few pustules on a few old live leaves, but most of the infected leaves were dead. The new leaves upon these plants were free from the rust. Upon the dry, dead leaves of these plants green areas surrounding the pustules were still evident. The tissue surrounding these areas had the brown appearance of tissue whose cells had disintegrated before they had dried. This would indicate that the green areas surrounding the pustules had died because of the drying out rather than because of the effect of the rust. The plants in wet soil and saturated atmosphere still had a number of live leaves heavily infected with rust. Some of the leaves however were dead or dying. On the latter, the infected areas remained green surrounded by yellow, sickly tissue or by brown tissue composed of dead cells. The new leaves had a small number of pustules showing that by this time some reinfection had taken place.

The development of *Puccinia Sorghi*, as shown by the number of pustules produced, is thus favored by the saturated atmosphere on the one hand and by the wet soil on the other. The length of the incubation period, however, is not much influenced. In dry air, the plants finally become free from the rust by the drying up of the infected leaves; and reinfection does not take place since spore germination is prevented in dry air. In a saturated atmosphere, the infected leaves live for a longer time since they do not dry up; and reinfection also takes place to some extent, since the spores produced are able to germinate in the humid atmosphere.

Effect of Mineral Salts upon Development

Two experiments were carried out to discover the effect of mineral salts upon the development of *Puccinia Sorghi.* In the first, water cultures were used, while in the second pure quartz sand watered with the solutions was employed.

Experiments 6 and 7.—The solutions used in the first of the two were Knop's full mineral nutrient solution, as given by Jost (1907) and Miss Wuist (1913), and solutions in which one of each of the eight principal elements (Mg, Ca, K, Fe, S, N, P, and Cl) were lacking. The full nutrient solution consisted of the following in 1,000 cc. of distilled water:

$MgSO_4$	.25 g.
$Ca(NO_3)_2$	1.00 g.
KH_2PO_4	.25 g.
KCl	.12 g.
$FeCl_3$	trace.

The nutrient solution minus calcium was made by substituting KNO_3 for $Ca(NO_3)_2$. K_2SO_4 was substituted for $MgSO_4$ to form a solution minus Mg. $Ca(H_2PO_4)_2$ and $MgCl_2$ were substituted for KH_2PO_4 and KCl to form a solution minus K. $MgCl_2$ was substituted for $MgSO_4$ to form a solution minus S. $Ca(H_2PO_4)_2$ was used in place of $Ca(NO_3)_2$ to give a solution minus N. KNO_3 was used in place of KH_2PO_4 to give a solution minus P. KNO_3 and $FePO_4$ were used in place of KCl and $FeCl_3$ to give a solution minus Cl. And $FeCl_3$ was left out of the full solution to form a solution minus Fe.

In the second experiment, maize was planted in quartz sand which had been thoroughly washed with distilled water and then dried. Knop's full mineral nutrient solution of three times the ordinary strength was used. The other nutrient solutions were made up a little different than in the preceding experiment in that, when an element was omitted, the concentration of the other elements in the solution was maintained except for the element it was replaced by. Thus for example, in −Mg solutions .75 gm. of $MgSO_4$ was replaced by 1.04 gm. K_2SO_4 so that there was as much SO_4 present as before. The amount of the replacing element, K, naturally increases. In the −Ca solution, $Ca(NO_3)_2$ was replaced by $Mg(NO_3)_2$ and KNO_3 so that there would not be too great a preponderance of either Mg or K in the solution.

Kernels of corn were planted in battery jars containing 1.2 kg. of quartz sand prepared as above and the jars were then placed in the greenhouse. The various solutions were used to water the plants. After 35 days, the plants were inoculated and placed in a moist chamber at 20° C. for twelve hours.

In both experiments sections through the infected areas showed that from about .5–1.2 mm. on each side of the pustules, there was no starch in the parenchyma sheaths, although it was present in considerable quantities in the rest of the leaf. Beyond this for about .2–.6 mm. on each side, the concentration of the starch gradually increased until it reached the full concentration of the rest of the leaf.

TABLE I

Effect of Mineral Starvation upon the Development of Puccinia Sorghi

Solution	Condition of Plants		Number of Plants Used		Number of Plants Infected		Average No. Pustules per Plant
	Water Culture	Sand Culture	Water Culture	Sand Culture	Water Culture	Sand Culture	Sand Culture
Full solution. .	green	green	2	5	2	4	197
— Ca.	yellowish	dead	3	5	1	0	0
— Fe.	"	light green	4	5	4	4	31
— Cl.	"	" "	4	5	4	3	170
— Mg.	"	" "	4	5	4	2	8
— K.	green	" "	4	5	3	3	32
— P.	dark green	" "	4	5	4	2	12
— N.	light "	" "	3	5	3	3	11
— S.	green	" "	4	5	3	2	13

The results as given in Table I show that mineral starvation does not prevent infection of *Puccinia Sorghi* but only that the amount of rust as shown by the number of pustules is less. Starch is prevented from forming in the immediate vicinity of the pustules.

EFFECT OF LIGHT UPON DEVELOPMENT

The effect of light upon development of the rusts was studied in a set of experiments, the results of which are given below. A number of pots of the plants were inoculated under the same conditions Some were then placed under belljars in the light, and the rest were covered with dark cylinders. After a few days, the plants under the dark cylinders were placed in the light and the incubation periods of each recorded.

Puccinia Coronata

Experiments 8, 9, and 10.—In these experiments inoculation was accomplished as stated above and the results obtained are given in the following table.

Table II

Effect of Light upon Development of Puccinia Coronata

Experiment	Pot No.	Time in Dark	Time in Light	Incubation	Retardation
8	C 17.1		10 days	10 days	
	C 17.2		10 "	10 "	
	C 17.3	5 days	10 "	15 "	5 days
	C 17.4	5 "	10 "	15 "	5 "
	C 17.5	18 "		died, no infection	
	C 17.6	18 "		died, no infection	
9	C 17.7		10 "	7–10 days	
	C 17.8		10 "	7–10 "	
	C 17.9	7 "	6 "	12–13 "	6–7 "
	C 17.10	7 "	6 "	12–13 "	6–7 "
	C 17.11	20 "		no infection	
	C 17.12	20 "		" "	
10	C 17.13		10 "	10 days	
	C 17.14		11 "	11 "	
	C 17.15	7 "	5 "	13 "	2–3 "
	C 17.16	7 "	5 "	13 "	2–3 "
	C 17.17	20 "		no infection	
	C 17.18	20 "		" "	

The results of these experiments show that in the absence of light the development of *Puccinia coronata* is retarded and if left in darkness too long, the rust is killed.

Experiment 11.—In the preceding experiments, the plants were all placed in a dark moist chamber after spraying with the spore suspension. Fromme (1915) and I (1915) have found that the germ-tubes of the uredospores of this rust are negatively heliotropic. It seems possible from this that the retardation of the appearance of pustules might have been due to a failure of the germ-tubes to enter the host while in the dark. Then, when brought into the light, inoculation might have taken place from spores whose germination had been delayed. To test this a fourth experiment was set up in which some of the plants, after being sprayed with the spore suspension, were left under belljars in the light for from one to five days and were then placed under dark cylinders for various periods, after which some of them were returned to the light. After spraying with the spores, others were kept in the dark for various periods of time and were then brought into the light.

TABLE III

Effect of Light Upon the Development of Puccinia Coronata. Comparison of Plants Inoculated in Light and in Darkness

Pot No.	Time in Light	Time in Dark	2d Period Time in Light	Incubation Period	Retardation
C 17.19.....	1 day	4 days	6 days	11 days	2 days
C 17.20.....	1 "	4 "	6 "	11 "	2 "
C 17.21.....	1 "	19+ "		no infection	
C 17.22.....	5 "	15+ "		" "	
C 17.23.....	5 "	6 "	3 "	14 days	5 "
C 17.24.....	5 "	6 "	3 "	14 "	5 "
C 17.25.....	9 "			9 "	
C 17.26.....	9 "			9 "	
C 17.27.....	9 "			9 "	
C 17.28.....		5 "	7 "	12 "	3 "
C 17.29.....		1 "	8 "	9 "	
C 17.30.....		1 "	8 "	9 "	
C 17.31.....		5 "	6 "	11 "	2 "
C 17.32.....		20+ "		no infection	
C 17.33.....		1 "	8 "	9 days	
C 17.34.....		5 "	7 "	12 "	3 "
C 17.35.....		11 "	10 "	21 "	12 "
C 17.36.....		5 "	7 "	12 "	3 "

Following this experiment, plants were inoculated in areas marked with India ink and covered with dark cylinders. After two days, the inoculated areas were sectioned and the sections treated with chloral hydrate and iodine. Mycelium was found in some of the inoculated areas, but had not developed to a very great extent.

From these results it is evident that infection of *Avena sativa* by *Puccinia coronata* takes place in darkness as well as in light, although apparently the amount of infection is less in darkness.

Puccinia Sorghi

The first experiments upon the effect of light upon the development of *Puccinia Sorghi* were carried out in the same manner as with *Puccinia coronata.* The results obtained in these first experiments (Experiments 12–19) were not as clear cut as those obtained with the latter. Seven out of eleven plants which were in the dark three to eight days before being placed in the light had their incubation period lengthened one to two days. The other four had no retardation of their incubation period Four out of thirteen plants which were in the dark throughout the experiment became infected. Their incubation period was lengthened only two days. The remaining nine of the thirteen, however, remained uninfected.

Experiment 20.—In this experiment the procedure was the same as in the previous experiments, except that the plants were kept in the dark for five days to exhaust them as much as possible of carbohydrates. In this case, two out of the seven plants which were put in the dark for seven days did not have their incubation period lengthened at all. The other five had their incubation period lengthened from 2 to 4 days, which is shorter than the time they were in the dark. Infections occurred on two out of twelve of the plants in the dark for the entire time and in these cases the incubation period was lengthened. Since these results more nearly agree with those obtained with oats (*Avena sativa*), it would appear that the reserve food supply of the maize is to be considered as the cause of the disagreement. In the case of maize, the endosperm furnishes considerable food to the plant during the first month and by the time this is exhausted, the plant is of such a size that considerable reserve food is stored up in the stem and other organs of the plant. The next experiment was carried out with the object of exhausting this reserve as nearly as possible before inoculating.

Experiment 21.—In this experiment, young plants were used. In order to control the reserve food supply of the host as much as possible, maize was germinated in a moist chamber and after four days the endosperms were dissected away. The plants were then planted in quartz sand which had been moistened with Knop's solution and were left in the light until the leaves were out and the plants had taken on a green color. Following this they were removed to the dark for three days to exhaust the carbohydrates manufactured during this time. All were finally inoculated with uredospores of *Puccinia Sorghi* and four kept under belljars and eight under dark cylinders. Five of the latter were removed at various intervals and placed under belljars. Three were left under dark cylinders throughout the experiment.

From the results of this experiment, it is evident that when the reserve food supplies of the host are cut down to the minimum, the incubation period of the rust is lengthened to a period corresponding to the time that the host was placed in the dark and that when the host is kept in the dark, there is no development of the rust. Not only does this indicate a direct relation to the carbohydrate supply

TABLE IV

Effect of Light Upon Development of Puccinia Sorghi. Plants Exhausted as Nearly as Possible of Soluble Carbohydrates

Plant No.	Age of Plant	Endosperm	Time in Dark	Time in Light	Incubation Period	Retardation
FC–E 50....	13 days	without		5 days (died)	no infection	
FC–E 51....	13 "	"		7 days	7 days	
FC–E 52....	13 "	"		7 "	7 "	
FC–E 55....	13 "	"		8 "	8 "	
FC–E 54....	13 "	"	3 days	7 "	10 "	3 days
FC–E 58....	13 "	"	3 "	6 "	9 "	2 "
FC–E 59....	13 "	"	3 "	7 "	10 "	3 "
FC–E 56....	13 "	"	6 "	7 " (died)	no infection	
FC–E 60....	13 "	"	6 "	6 days	12 days	5 "
FC–E 61....	13 "	"	6 " (died)		no infection	
FC–E 53....	13 "	"	10 days		" "	
FC–E 57....	13 "	"	13 "		" "	

of the host, but the apparent exceptions in the previous experiments due to the presence of reserve food in the host only strengthen this conclusion the more.

EFFECT OF THE LACK OF CARBON DIOXIDE UPON DEVELOPMENT

The preliminary experiments to show the effect of the lack of carbon dioxide upon development were not satisfactory, since the plants used possessed an endosperm and derived their food supply from it as was shown by the plants in a minus carbon dioxide atmosphere developing as well as those of the check. Infection occurred at the same time as in the checks or a few days later. Since the experiments with light, which were being run at the same time, pointed to carbohydrates as factors in the development of the rusts, it was evident that the plant must be deprived as nearly as possible of carbohydrates. This was attempted in two experiments.

Maize was germinated in a moist chamber at 30° C. and at the end of six days, when the plumule had reached the length of five to six centimeters, the endosperm was dissected away and the plants were planted in small bottles filled with quartz sand and moistened with Knop's solution. The plants were grown in the light for a few days until the leaves were expanded and chlorophyll had developed. They were then placed in a dark chamber for three days to exhaust them of the carbohydrates which had been formed while in the light. Following this, the plants were inoculated with uredospores of *Puccinia Sorghi* and placed in large-mouthed liter bottles.

A carbon-dioxide-free atmosphere was obtained in these bottles by placing a strong solution of potassium hydroxide in the bottom of each and the oxygen supply was maintained by connecting the bottles with U tubes which contained a mixture of pumice-stone moistened with a KOH solution and pieces of KOH. Checks were run which were set up similarly, with the exception that KOH was omitted (Plate IV, figure 1). All the joints and corks were coated with paraffine.

Two experiments were conducted in this manner, the results of which are given below.

Experiment 22.—In the first experiment, plants treated as above were inoculated and six (FC–E 60–65) were placed in bottles having a carbon-dioxide-free atmosphere and two (FC–E 66 and 67) in check bottles. The set was kept in the dark for 24 hours to allow the apparatus to become free from carbon dioxide and then was placed in the light of an east window at an average temperature of 22° C. In four days, numbers FC–E 60–65 began to show the effects of the lack of carbon dioxide by their sickly appearance. Numbers FC–E 66 and 67 remained fresh and healthy. Pustules first showed on Numbers FC–E 66 and 67 in eight days. The plants in carbon-dioxide-free atmosphere at this time showed no signs of infection. Numbers FC–E 64 and 65 were dead and the upper parts of the leaves of Numbers FC–E 60–63 were also dead.

The experiment was finished on the eleventh day. At this time no infection had taken place upon any of the plants in the carbon-dioxide-free atmosphere.

Experiment 23.—Eight plants (FC–E 68–75) which were treated as in the preceding experiment were inoculated with spores and placed under a dark cylinder for 12 hours at 18° C. Six of these (FC–E 68–73) were then placed in bottles with a carbon-dioxide-free atmosphere and two (FC–E 74, 75) were used as checks. The bottles were all kept in the dark for 12 hours so that the plants might not manufacture carbohydrates while containing carbon dioxide. Pustules appeared on both the checks in six days and on the seventh day pustules appeared on FC–E 69 and 73 in small numbers. These two plants, however, appeared fully as healthy in every way as the checks, while FC–E

68, 70, 71, 72 plainly showed the lack of carbon dioxide in their lighter green leaves, which were dying back from the tip.

The experiment was finished at the end of the twelfth day. There was no infection on FC–E 68, 70, 71, 72, which, although they showed indications of approaching death, were still alive. Not even greenish spots showed on the yellowing leaves such as often appear upon dying leaves in infected areas. FC–E 69 and 73 at this time had a number of open pustules and the plants themselves were in every particular as healthy as the checks, showing that the apparatus in these cases was defective and did not eliminate entirely all the carbon dioxide, or that the plants possessed a large enough amount of food at the beginning of the experiment to supply them.

These two experiments show, as do those with light, that there is a relation between the development of the rust and the carbohydrate supply.

Effect of Supplied Carbohydrates upon Development

Since the preceding work indicated a relationship of the rust to the carbohydrate supply, further experiments were undertaken in order to study this relationship more thoroughly. To do this it is necessary to supply carbohydrates to the host which has been deprived of them as nearly as possible, since the experiments with light indicate that the host normally contains more or less carbohydrates in available form for the use of the rust.

This work divides itself into two parts according to the manner of supplying the carbohydrates to the host. In the first case, carbohydrates were supplied to the plant through the scutellum of the maize seedling and through the roots. Van Tieghem (1873) has shown that embryos can be developed upon starch. Brown and Morris (1890) have found that not only do excised embryos develop normally upon starch, but that they will also do so with sugar solutions, especially cane sugar, and that when both starch and sugar are present, the sugar is used up before the starch is attacked.

A number of authors have investigated the ability of plants to take up carbohydrates through their roots. Mazé and Perrier (1904) obtained a good growth of maize in 1 percent glucose and sucrose. Acton (1889) found that in this way acrolein, acrolein-ammonia, allyl alcohol, glucose, acetic aldehyde, ammonia, glycerine, laevulenic acid, calcium laevulinate, cane sugar, inulin, dextrins, glycogen, and extract of natural humus can be used by a number of plants when in a carbon-dioxide-free atmosphere. J. Laurent (1897, 1898, 1904) investigated this subject thoroughly for corn. He found that glucose, invert sugar, sucrose, soluble starch, and starch can be taken up by corn through its roots and utilized in a carbon-dioxide-free atmosphere and to a somewhat less degree in a normal atmosphere in the dark.[2]

In the second case, cut pieces of leaf were floated upon various carbohydrate solutions. A number of workers have shown that portions of plants may utilize sugar from solutions in which they were placed. Boehm (1883) showed that cut pieces of leaf of *Phaseolus multiflorus* upon solutions of cane sugar and glucose form starch in the dark. E. Laurent (1886) has shown that etiolated potato sprouts placed in the dark with their cut ends in a 10 percent cane-sugar solution grow for more than five months and form starch. A. Meyer, according to Acton (1889), found that shoots, when supplied with dextrose, glycerine, sucrose, and inulin, can form starch. A number of other authors have carried out similar experiments with like results.

Carbohydrates Supplied to Seedlings

In order to grow plants in carbohydrate nutritive solutions, it is necessary to have both the plants and solutions as nearly sterile as possible or the solution will be quickly filled with the growth of a number of saprophytic fungi. The only feasible way to obtain sterile seedlings is to sterilize the seed. A number of methods have been used by different workers for sterilizing seeds, but almost all of these methods have been found inefficient in some particular by other workers.

Ward (1902*d*) used "various antiseptics" and also heated the seeds to 60–70° C. for the purpose of sterilizing brome seeds. These were then placed in sterile petri-dishes to germinate. Laurent (1897, 1904) used .2 percent $HgCl_2$ solution for 1½ to 3 hours for corn. J. K. Wilson (1915) has recently reported good results for a number of seeds from the use of a solution of chlorine obtained from bleaching powder. A number of other antiseptics have been used, such as $H_2 SO_4$, $CuSO_4$, H_2O_2, phenol, HNO_3, etc., none of which have been found generally or uniformly successful. Of these H_2SO_4, $HgCl_2$, and the calcium hypochlorite method of Wilson were tried by myself.

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[2] Since the above was written Kundson (1916) has also shown that maize is able to take up through its roots dextrose, laevulose, maltose, and sucrose from their 2 percent solutions with an increase in growth and dry weight.

In the use of the first, grains of yellow dent corn were cleaned and dropped into concentrated H_2SO_4 for ten minutes. They were then removed to a capsule of sterile distilled water and washed once or twice with sterile distilled water. They were then placed in sterile, moist chambers. This treatment with H_2SO_4 did not appear to injure the seed in any way. On the other hand, it hastened germination of the seed. But it was not effective in killing the particular fungus spores which were present on these occasions and in nearly every case, fungi developed in the moist chambers.

The use of $HgCl_2$ was found to be much more satisfactory and it has been used entirely in this work where sterile corn plants were needed. The method used was to clean the seeds and drop them into .5 percent $HgCl_2$ solution where they were left for thirty minutes. The $HgCl_2$ solution was then poured off and replaced several times with sterile distilled water. The corn was then removed with sterile forceps and placed in large (4.3 × 25 cm.) sterile test tubes, which were placed in an incubator at 27°–30° C. This method gave very good results throughout the work and only a small number of contaminated seedlings were found.

Wilson's calcium hypochlorite method was tried, but it did not give very good results. Ten grams of "Acme" chloride of lime (bleaching powder) containing 30 percent chlorine were mixed with 140 cc. of water according to Wilson's directions and maize was treated for 9 hours with the filtrate. Only very slight germination and poor seedlings were obtained by this treatment. The corn used however was a little over a year old and this may account for the failure, although it gave very good germination when treated with $HgCl_2$, as stated above. To test this method still further corn was taken out of the sterilizing solution every hour for eight hours and placed in sterile moist chambers and then placed in an incubator at 27° C. Good germination took place with corn treated for one and two hours, but when treated beyond that time the germination was poor. All the moist chambers contained more or less contaminated seedlings. This method when used with oats and wheat gave good results, while $HgCl_2$ as outlined above was unsatisfactory with oats and wheat.

Although the number of disinfecting solutions used was not very great, yet the results obtained point to the uselessness of trying to obtain a disinfectant which will work for all seeds under all conditions. Not only will the kind of seed but also the age of the seed, the amount of water contained in it, the permeability of the seed coat, and the kind of fungus spores on it—all variable factors—have important bearing upon the effect of sterilizing solutions upon the seed. It is very unlikely that any one solution will work effectively under all combinations of these conditions. Such a situation means that each worker must select the agent which is the best suited to meet the requirements of his particular conditions.

The corn after having been sterilized with $HgCl_2$ was germinated in large sterile test tubes. The necessary moisture was maintained in the tube by having absorbent paper saturated with distilled water in the bottom of the tube when sterilized. The tubes were kept in the dark in incubators at a temperature of 27°–30° C. for about seven days, at which time the plumule of the corn had attained the height of 5–15 cm.

The endosperm of corn contains a quantity of nutriment which can nourish the plant for about a month and in fact when the plant is grown in the light, the endosperm often lasts much longer, even two months. It is therefore necessary to remove the endosperm before placing the plants in nutrient solutions, since it would furnish all the plants of the experiment with a large carbohydrate supply. This is done by removing the plant from the test tube with sterile forceps and making a longitudinal cut through the endosperm down to the scutellum. The action of diastase has by this time dissolved away the portion of the endosperm lying next to the scutellum and the two halves of the endosperm are easily removed with sterile forceps, leaving the scutellum surface exposed. The plants are then placed in their nutrient solutions.

The following solutions were used: Cane sugar 15, 12, 6, and 3 percent; cane sugar 10 and 3 percent plus Knop's mineral nutrient; cane sugar 10 percent plus Knop's mineral nutrient minus nitrogen (see Experiment 6); starch jelly 15 percent; starch jelly 15 percent plus Knop's mineral nutrient; starch jelly 15 percent plus Knop's mineral nutrient minus nitrogen; dextrose 3 percent; dextrose 3 percent plus Knop's mineral nutrient; maltose 3 percent; maltose 3 percent plus Knop's mineral nutrient; dextrin 3 percent; dextrin 3 percent plus Knop's mineral nutrient; Knop's mineral nutrient; Knop's mineral nutrient minus nitrogen; distilled water.

Erlenmeyer flasks of 150 cc. capacity were used to contain the solutions. These were stoppered with cotton plugs and autoclaved at 110° C. for 30 minutes. It was found that there was much less contamination of cultures if the flasks were autoclaved just before using and allowed to cool in the autoclave. If this was not possible, the flasks were usually wiped off with a corrosive sublimate solution before using, since the roots of the corn plants often touched the outside of the flasks while they were being placed in the solution due to the small neck of the flask. The cotton plugs used to stopper the flasks were replaced after flaming and as they were made rather loose and somewhat larger than the necks of the flasks, they fitted rather closely around the corn stems (Plate IV, figure 2).

The flasks with plants thus prepared were placed in a moist dark chamber. This chamber was prepared by covering a galvanized tank (1¼ × 1 × 3 ft.) with a cover made of heavy black paper. The cover was a little larger than the tank and reached to the bottom on the sides, so that light was excluded and ventilation permitted. A layer of water was kept in the bottom of the tank while the work was being carried on in the greenhouse; this was enough to maintain a saturated atmosphere in the chamber. Later when the experiments were conducted in the drier air of the laboratory, coarse woven cloth which was kept wet was spread over the tank under the black paper lid in order to maintain the saturated atmosphere.

After the plants had been in this dark chamber for several days, they were inoculated by spraying with a spore suspension and in addition a small quantity of spores was placed on certain leaves. They were kept at 20° C. for 24 hours, at which temperature the uredospores of *Puccinia Sorghi* germinate vigorously.

The results of Experiments 24 to 31 are given in the following table. In all the cases where infection took place upon plants in Knop's nutrient solution or distilled water, the pustules were poorly developed and few in number. In the three experiments where such infection occurred, the infected portion of the leaves were cut off and the plants were reinoculated. No infection took place the second time on such plants in distilled water or Knop's nutrient. Plants in sugar solution, however, were infected, although to a somewhat less extent than in the previous experiments. The reinfected plants were left until all of them died. It was found that the plants which were infected in Knop's and distilled water in the original experiments lived as a rule longer than the others. This would indicate that infection in the original experiments upon these plants was due to a supply of food

TABLE V

Effect of Carbohydrate Supplied to the Seedling Upon the Development of Puccinia Sorghi

Solution	No. of Plants Used	No. of Plants Infected	Av. Growth per Plant per Day
Starch 15%	10	9	3.4 mm.
Starch 15% + Knop's	10	3	1.6 "
Starch 15% + Knop's − N	10	5	5.1 "
Cane sugar 15%	12	3	.9 "
Cane sugar 12%	6	3	3.7 "
Cane sugar 10% + Knop's	12	6	3.3 "
Cane sugar 10% + Knop's − N	13	5	3.7 "
Cane sugar 6%	6	4	6.0 "
Cane sugar 3%	21	13	7.6 "
Cane sugar 3% + Knop's	21	13	11.3 "
Maltose 3%	7	5	4.9 "
Maltose 3% + Knop's	7	4	9.1 "
Dextrin 3%	6	3	11.0 "
Dextrin 3% + Knop's	7	2	12.6 "
Dextrose 3%	18	5	4.8 "
Dextrose 3% + Knop's	18	9	9.8 "
Knop's nutrient	50	3	2.6 "
Knop's nutrient − N	13	0	.4 "
Distilled water	45	8	2.3 "

present in the host upon which the rust as well as the host was able to draw. When reinoculated this was exhausted and there was no infection of hosts in either distilled water or Knop's solution. The results of these experiments indicate that soluble carbohydrates are necessary for the development of the rust.

CARBOHYDRATES SUPPLIED TO PIECES OF LEAF

In the earlier part of this work contaminations occurred which were due to working with imperfectly sterilized leaves and especially with rust spores having saprophytic fungus spores mixed with them.

It was evident that to obtain trustworthy results not only sterile host plants were necessary, but that pure cultures of the rust must also be obtained. This was done as follows:

Pure Cultures of Puccinia Sorghi

The only worker who has given any account of a method to grow rusts in pure culture appears to be Marshall Ward (1902*d*) working with *Puccinia dispersa* upon the bromes. His method consisted in obtaining sterile cultures of the bromes by sterilizing the seed by "steeping in various antiseptics, or by heating to 60–70° C." The sterile seeds were placed in sterile drying towers, supplied with Knop's mineral solution and aerated with a continuous current of air or were placed in large sterile test tubes which contained the solution. When the first leaf was well developed it was inoculated with uredospores of *Puccinia dispersa*. Good infection was obtained in the inoculated area.

Ward's object in developing this method was to be sure he was working with only one race of *Puccinia dispersa* and not so much to free the rust from saprophytic fungus spores. He does not say that the spores with which he inoculated his sterile plants were free from other fungus spores yet he assumes that he had a pure culture as far as fungi were concerned. He says (p. 459), however, that the method does not exclude harmless bacteria. In order to be sure that spores of saprophytic fungi were not present in the sowing of the rust spores, it would have been necessary to sow the spores of the resulting rust upon nutrient media. This is necessary, since many saprophytic fungus spores do not germinate except in the presence of such nutrient media and so would remain dormant upon the surface of the plant in the infected area and be removed with the spores of the resulting rust.

Two methods have been developed for obtaining pure cultures of the rust. The first method is a modification of Ward's. Large test tubes (30 × 5 cm.) were prepared by filling the lower end with absorbent paper and adding Knop's mineral solution. These were then stoppered with cotton plugs and autoclaved at 10 pounds pressure for 30 minutes. Two or three seeds sterilized in .5 percent $HgCl_2$ solution for 30 minutes were placed in each test tube and the tubes placed in a well lighted window. After the seedlings had developed one or two leaves, they were inoculated with uredospores of *Puccinia Sorghi* (Plate V, figure 1).

The uredospores were obtained from well-developed pustules of *Puccinia Sorghi* and were placed in a capsule of sterile distilled water and thoroughly mixed up. The uredospores of *Puccinia Sorghi*, as well as most other rusts, are much lighter than water and float on the surface, from which they can be removed with a looped platinum wire and placed upon the leaves of the corn. The first trials to obtain infection in this way were failures because of the lack of adhesion of the water drops to the waxy surface of the corn leaves. In ordinary infection work, the leaves are gently drawn between the fingers before inoculation to remove this waxy substance. Since this method would cause contamination in this case, other means were resorted to. It was noticed that drops of water often condensed upon the leaves during cool nights. Spores were placed in these with resulting infection. As carried out later, the leaves were rubbed with sterile cotton wrapped around sterile forceps and soaked in sterile distilled water. In the drops left adhering to the surface of the leaves, a loop full of uredospores was placed. In this way very good infection was obtained. In most cases, the spores used, after the combined dilution and washing to which they were subjected, were probably sterile, but in order to make sure that there was no contamination, spores from the resulting infection upon these plants were used to inoculate other sterile plants. These spores were taken from the other side of the leaf from that on which the original inoculation had been made. By this means they were obtained from a sterile surface which had not been touched in the original inoculation. The plants of this second series, when once infected, will be reinfected by the spores produced on them as long as they are in good condition, if kept at a temperature favorable for spore germination (Plate V, figure 1).

A second means of obtaining pure cultures of *Puccinia Sorghi* was by means of cut pieces of leaf themselves. Uredospores which had been removed from clean parts of infected plants were diluted in sterile distilled water. Drops from this spore dilution were placed on the surface of pieces of leaf which had been cut from sterile corn plants, and floated upon carbohydrate solutions. A few capsules were contaminated by saprophytic fungi, but more often capsules were obtained which were free from these and the rust produced in such capsules was used to inoculate pieces of leaf in like manner. By inoculating fresh cultures about every two or three weeks, a pure culture of the rust can be kept on hand (Plate V, figure 2).

Experiments 32–41.—These experiments were carried out with pure cultures of both host and rust. Cut pieces of sterile corn leaf were floated upon sterile solutions of 3 and 6 percent cane sugar, 3 percent cane sugar plus Knop's, 3 percent dextrose, 3 percent dextrose plus Knop's, 3 percent maltose, 3 percent maltose plus Knop's, Knop's, and distilled water. After one to three days, uredospores from pure cultures of *Puccinia Sorghi* upon sterile plants were diluted in sterile distilled water and a drop of this spore suspension was placed on each piece of leaf. The capsules were placed in a dark chamber at 20° C.

The results of these experiments are given in the following table.

TABLE VI

Effect of Carbohydrates Supplied to Cut Pieces of Leaf Upon the Development of Puccinia Sorghi

Solution	No. of Pieces of Leaf Used	No. of Pieces Infected	Remarks
Cane sugar 6%.................	12	6	pustules large
Cane sugar 3%.................	31	13	" "
Cane sugar 3% + Knop's........	36	8	" "
Dextrose 3%...................	7	3	" "
Dextrose 3% + Knop's..........	7	4	" "
Maltose 3%....................	9	5	" "
Maltose 3% + Knop's...........	10	2	" "
Knop's nutrient...............	30	2	pustules few, small
Distilled water...............	30	1	3 pustules, small

Of the pieces of leaf on Knop's nutrient solution which were infected, one piece had one very small pustule, while the other had three very small pustules which were light in color, very unlike the brown color of a vigorous rust. The infection upon carbohydrate solutions varied from a few large brown pustules to a mass of pustules which covered nearly all the area of the piece of leaf (Plate V, figure 2). All the pieces of leaf which were on carbohydrate solutions had the cells of the mesophyll and parenchyma sheaths filled with starch. Pieces of leaf on Knop's solution and distilled water showed no sign of starch. At the end of 14 days, most of the pieces of leaf were alive, as was shown by plasmolyzing with strong KNO_3.

These results agree with those obtained with plants in nutrient solution.

EFFECT OF NUTRITIVE SOLUTIONS UPON SPORE GERMINATION AND CONTINUANCE OF GROWTH

In this work, the uredospores of *Puccinia Sorghi* were sown upon a number of different nutritive solutions. Compounds were used which it was thought would likely be utilized by the rust in its host.

The method employed in the first two experiments was to remove uredospores from an infected plant as carefully as possible and float them on the surface of a sterile solution of the nutrient material to be used. Hanging drops of this spore suspension were then made.

In order to avoid the work necessary for making a large number of van Tieghem cells, the hanging drops were made upon the lid of a sterile Petri dish. Evaporation from the drops was prevented by either having absorbent paper moistened with the nutrient solution used in the bottom of the dish or by having a small amount of the solution alone. If absorbent paper was used, a V-shaped piece was cut out so that the microscope could be used to observe the development of the rust in the hanging drops, each of which in turn could be brought over the V-shaped opening by turning the cover upon the bottom part of the dish.

The germination could be watched under the 16 and 8 mm. objectives with clearness and the growth and condition of the germ-tubes could be easily followed. Each Petri dish had ten hanging drops on its lid and since three Petri dishes were used for each solution thirty hanging drops were employed for each nutrient medium.

Some contamination resulted in these cultures, but most of the hanging drops showed only a slight growth of saprophytic fungi during the short time that cultures were run.

Experiment 41.—The nutrient media used in this experiment were conductivity water, cane sugar 1 percent, cane sugar 5 percent, maltose 1 percent, maltose 5 percent, leucine 1 percent, asparagine saturated solution, asparagine 1 percent and peptone (Witte's) 1 percent. Besides these, a mineral solution, and carbohydrates plus the mineral solution were used. The mineral solution used was Duggar's standard nutrient solution (1909) for fungi minus the sugar. It consisted of the following, dissolved in 100 cc. of water:

NH_4NO_3	1.00 gm.
KH_2PO_4	.5 gm.
$MgSO_4$	.25 gm.
$FeCl_3$	trace.

The cultures were kept at 17° C. during the experiment. The following table gives the results of the experiment.

Since a dense mass of hyphae was produced during the germination of the spores, the number of germinated spores could not be accurately counted and the amount of germination was estimated by the appearance. In the column under remarks the germ-tube is described whether it produced short side branches or was unbranched. In all these solutions, the rust was dead in about four days.

Experiment 42.—Since the preceding experiment indicated that strong concentrations were injurious to spore germination and that

Table VII

The Effect of Nutritive Solutions Upon Spore Germination and Continuance of Growth of Puccinia Sorghi

Solution	Germination	Length of Germ-tube	Remarks
Conductivity water	good	400–500 micr.	unbranched
Mineral nutrient	slight	160 "	"
Cane sugar 5%	fair	160–800 "	"
Cane sugar 1%	good	400–800 "	"
Cane sugar 5% + nut	none		
Maltose 5%	fair	about 100 "	"
Maltose 1%	good	300–800 "	branched some
Maltose 5% + nut	very slight	about 100 "	unbranched
Peptone 1%	fair	" 500 "	branched much
Peptone 1% + nut	none		
Duggar's nut + .5% peptone	none		
Asparagine saturated	slight	" 160 "	branched
Asparagine 1%	fair	80–100 "	"
Leucine 1%	slight	100–400 "	" slightly

they shortened the length of the germ-tube, an experiment was undertaken to study the germination and development of the rust at low concentrations. Two solutions, 1 percent cane sugar and 1 percent cane sugar plus mineral nutrient (see Experiment 41) were used as the basis of this series. Dilutions of 1/2, 1/8, 1/32, 1/128, 1/512, and 1/1024 of this strength were made. Hanging drops of uredospores of *Puccinia Sorghi* were made in these solutions as in the preceding experiment.

No difference was noticed in the amount of germination in these solutions. The length of the germ tube was the greatest (400–800 micr.) in 1/8, 1/2 and 1 percent cane sugar. In the other solutions, the length varied between 160–400 micr. The rust died in all solutions in about three days.

Experiment 43.—In this experiment, plant extracts were used. A decoction of leaves of corn was made by autoclaving pieces of corn plants (1 part by vol. to 5 parts of distilled water) at 10 pounds pressure for 30 minutes. An uncooked extract of the plant was made by cutting up sterile plants as finely as possible and adding sterile distilled water to them and then letting the mixture stand for 24 hours. A third extract was made from sterile germinated seeds which were cut up in sterile distilled water. Two or three seeds were used for 25 cc. of water. Uredospores of *Puccinia Sorghi* from pure cultures of the rust were sown in hanging drops and in capsules of the solutions.

The germination in all cases was good. In the decoction of the host, the tubes were long (about 800 micr.) and somewhat branched. In the other two solutions, the germ-tubes were long and abundantly branched. Death took place in all these solutions in about four days.

Experiment 44.—Cane sugar 3 percent, cane sugar 3 percent plus Knop's nutrient, dextrose 3 percent, dextrose 3 percent plus Knop's nutrient, Knop's nutrient, and distilled water were used in this experiment. Pieces of corn leaf were floated on these solutions and the solutions were then autoclaved for 30 minutes at 10 pounds pressure. This culture was run at the same time as Experiment 39 and inoculation with uredospores of the rust was made in the same way.

Infection took place upon the living leaves upon the carbohydrate solutions in Experiment 39 in eight days. No development of the rust occurred upon any of the autoclaved leaves.

IV. DISCUSSION

The first question of interest concerns itself with the condition of the tissues in and around the region invaded by the rust. In the development of *Puccinia Sorghi*, it is noticeable that, although most of the cells of the leaf may be invaded by the large haustoria, yet no harmful effect is shown by the host until after some period of time. The rust sends its mycelium through the intercellular spaces and then its haustoria into adjacent cells. The invaded cells retain the characteristics of cells of uninfected tissues. The first sign of effect upon the host is seen in the gradual disappearance of starch from the parenchyma sheaths in the invaded region. Since the parenchyma sheaths serve as a storehouse for the assimilated material from the adjacent region, and since they are not invaded for some time, it would appear that this loss of starch is due, not to a withdrawal of starch from the parenchyma sheath by the fungus itself, but to the utilization by the fungus of the material formed in the neighboring region before it reaches the parenchyma sheaths. That, even at this stage, the rust is not attacking the host vigorously is shown by the development of more or less starch in the parenchyma sheaths of the invaded region depending upon the conditions of photosynthesis at the time of observation. That the rust is having some effect is shown however by the paler color when the parenchyma sheaths of the infected areas are stained with iodine.

This condition prevails up to the time of spore formation. At this time, the rust begins to draw more heavily upon the host in order to obtain the necessary materials for spore formation. This is evident in the smaller amount of starch present in the parenchyma sheaths in the immediate region of the pustules. Oftentimes the parenchyma sheaths are here entirely devoid of starch, while in the neighboring region starch is present to a considerable extent. Yet even at this time, the cells of the host do not show an injury such as one would expect if the protoplasm itself was attached vigorously by the fungus.

It is only after the number of pustules have increased and spore formation has continued for some time that the host begins to show the effect of the rust's presence. The effect of the rust, even now, is not apparent in the tissues containing the rust, but in the neighboring tissues as is shown by the green color of the infected areas and the lighter green or yellow of the surrounding tissue. The green tissue of the infected areas even at this time, may contain small amounts of starch, but the neighboring dying regions have no indications of starch. It would thus appear that the rust instead of attacking and killing the cells of the tissue in which it is situated has a very different effect upon them. While it is withdrawing food, at the same time it stimulates the infected tissue so that this loss of food is in turn compensated by the withdrawal of food from neighboring uninfected tissue. It would appear that the rust thus destroys the symbiotic balance between the cells of the host and causes some of them to have parasitic relations with the rest. Marshall Ward (1902*b*) and Tubeuf (1897) observed this effect and considered it as evidence of a symbiotic relation between the rusts and their hosts.

As this withdrawal of food goes on the yellowing of the leaf extends farther and farther from the green infected area, the cells of the region gradually die and shrivel up, and the tissue takes on a brown appearance similar to that of cells which have died due to a decomposition of their contents. The infected areas, however, still remain alive for some time, but in these areas death results from two causes. The first of these is the cutting off of the food supply due to the death of the surrounding tissue. This, however, is not probably the principal cause as the green cells of the infected areas could furnish food to prolong their life and that of the rust until by a process of gradual starvation both would die. The principal cause which appears to bring about the death of these areas is the drying up of the leaf as a whole. As Sachs has pointed out the loss of water from dead tissue is much greater than from living. The great evaporation from the surrounding dead tissue naturally withdraws water from the green infected areas, which have their water supply from the root diminished, and brings about death through drying out.

If conditions at this time are unfavorable for spore germination, such as a low humidity or a too low or too high temperature, the corn plant will be freed from the rust, since the spores formed upon the old leaves will not be able to infect the young newly formed leaves. With the death of the old leaves the host becomes free from the rust. In the same way, oats may become free from their rust.

The work upon the effect of temperature upon the development of the rust also throws some light upon the relation between the rusts and their host. From Experiments 1 and 2 and especially 3 and 4, it is evident that in a saturated atmosphere the development of both *Puccinia coronata* and *Puccinia Sorghi* is retarded by low temperatures.

It is difficult to say just how much these results are due to the direct effect of the temperatures upon the rust, since the rust must be studied in connection with its host. A search of the literature shows that but little work has been done upon the effect of temperature upon the growth of parasitic fungi in their hosts. Sheldon (1902) found that during the winter months the incubation period of *Puccinia Asparagi* was longer than during the summer months when the temperature was higher. Fromme (1913) found a shorter incubation period for *Puccinia coronata* at temperatures between 20° and 30° C. than at lower. Ward (1902*b*) explains the non-infection in some of his experiments by the high temperature, although the host seemed to be unharmed.

The effect of temperature upon the development of saprophytic fungi has received considerable attention. In such experiments, however, the nutrient media remained unchanged. In experiments with the rusts, other conditions besides the temperature alter, since the physiological conditions in the host are altered. It is consequently hard to determine how much of the effect produced on the rust is due directly to the temperature. Lehenbauer (1914) has shown that for corn the optimum temperature for the growth was situated between 29 and 32° C. Sachs (1882) gives 27.2° C. as the optimum temperature for the growth of the root. Besides the effect upon the growth, two of the physiological processes of the host are especially affected. These are respiration and photosynthesis. The respiration of plants increases with the increase in temperature until the injurious effect of the high temperature brings about a disorganization of the vital functions of the plant. Photosynthesis according to Pfeffer (1900) increases with the temperature up to an optimum, which is approximate to that of growth and then it falls. Matthaei (1905), on the other hand, gives a curve for photosynthesis which resembles that of respiration. This curve, however, is a curve of maximal pnotosynthesis for each temperature. At high temperatures, the maximal photosynthesis is maintained only for a short time. The higher the temperature the sooner the decline sets in and the steeper its slope. The full development of photosynthesis also needs the best conditions of illumination while respiration is not much affected. Consequently in Experiment 4, the food supply available for the fungus at 30° C. is much reduced from that which would be available for it at 20° C., since photosynthesis has fallen off and respiration has increased. At the lower temperatures, although the respiration is lowered, photosynthesis is also reduced and to a much greater degree so that the food available for the fungus is less than at the optimum temperature for photosynthesis.

Although the growth of the fungus is thus influenced to some extent by the effect of temperature upon the amount of food supply available in the host, it is probably the direct effect of the temperature upon the rust itself which is the most important factor in determining the development of the rusts in Experiments 1–4; especially since the temperatures obtained for growth also correspond very well to those for spore germination. The actual temperature of the fungi in these experiments, however, was undoubtedly higher than that recorded, since Matthaei (1905), Ehlers (1915), and others have shown that the internal temperature of leaves is one to ten degrees higher than the surrounding atmosphere, depending upon the amount of illumination, the presence or absence of air currents, and the amount of transpiration.

The evidence concerning the effect of moisture upon the development of the rusts is rather conflicting. Blaringhem (1912, 1913) and Stone and Smith (1899) claim that the rusts are favored by a dry soil. Buchet (1913) believes that a wet soil is favorable. Sirrine (1900) considers that dew is the controlling factor. Smith (1904) finds that a dry atmosphere is unfavorable to *Puccinia Asparagi*, while a dry soil is favorable. From the results of Experiment 5, it is evident that for *Puccinia Sorghi* wet soil and moist atmosphere bring about an increase in vigor, as shown by the greater number of pustules and the consequently more abundant spores. A humid atmosphere also lessens the transpiration from the dying portions of the leaf and the evaporation from the dead areas so that the infected leaves in a moist atmosphere have a much longer life. In drier air, the infected leaves dry up and the plants become rust free.

The effect of mineral starvation upon the host has been investigated principally by Marshall Ward (1902*c*). Sheldon (1905) and McAlpine (1906) have made some observation upon the effect of soils and manures upon the development of some of the rusts.

Results similar to Marshall Ward's were obtained in Experiments 6 and 7. Infection was obtained upon some plants in all of the mineral nutrients. Table I shows that the best infection was obtained upon plants which were furnished with a full mineral nutrient. A deficiency of an element, however, does not bring about immunity for the host, but it causes a smaller number of plants to be infected and a lessening of the amount of rust as shown by the number of pustules.

That this effect is produced upon the rust is due partly to a lack of these elements for the host and a consequent slow mineral starvation of the rust. This, however, only explains a portion of the effect produced, since the rust can probably obtain these elements in small quantities from the host as long as the host is alive. A portion of the effect produced is at least to be referred to the effect upon the physiology of the host and its greatly reduced ability to manufacture the proper food materials for the rust. That this is true is shown in Table V. In this table it is seen that in solutions in which the host was supplied with a mineral nutrient solution, but was not supplied with carbohydrates and was kept in the dark to prevent their manufacture, the number of plants infected were few. That even this number was infected was due to the fact that the host was not exhausted of soluble carbohydrates, such as the sugars, before infection. The infection of plants supplied with carbohydrates in all cases far outnumber that of the plants without carbohydrates.

The need of carbohydrates is also shown in Experiments 8–21 conducted upon the effect of light upon the development of the rusts. Of the two rusts *Puccinia coronata* upon oats shows the closest relation in this regard. Thus, in Table II, it is shown that the retardation in infection approximates closely the period that the host was left in darkness and consequently the period during which carbohydrates were not being formed. In all cases where the host was kept continuously in darkness after inoculation there was no infection.

That these results are due to the prevention of photosynthesis and not either to non-inoculation due to the negative heliotropic germ-tube of *Puccinia coronata* or to the effect of the lack of light upon the development of the mycelium of the rust is shown in Experiment 11. Since it has been shown that the germ-tubes of the uredospores of *Puccinia coronata* are negatively heliotropic, the explanation of the retardation of the incubation period might be an inability of the germ-tube to enter the stoma while in the dark. This is not the case, as shown by the fact that when plants (C 17.19–C 17.27, Table III) after having been inoculated in the light were placed in the dark for a short period, the incubation period of the rust on them was also lengthened. The demonstration of the presence of mycelium in the leaves of plants inoculated and kept in the dark finally establishes this.

In Experiment 11 there is one case (C 17.35) where the retardation of the incubation period was greater than the time during which the host was in the dark. In this case, the oats were in the dark for eleven days and were consequently so starved and their physiological processes so disarranged that when returned to the light, they carried on their physiological processes poorly and so were able to furnish the rust with only a small amount of food.

The relation of *Puccinia Sorghi* to the carbohydrates formed in the light is not at first glance so striking as in the case of *Puccinia coronata*. Heckel (1912) and Blackshaw (1912) have however shown that corn plants contain from 6 to 9 percent of sugars. Besides this, corn forms starch in the parenchyma sheaths so that under ordinary conditions it contains quite a considerable reserve of carbohydrates. Oats, on the other hand, contain no such reserve and consequently the rust quickly shows the effect when the daily supply is cut off. When means were adopted to decrease the sugar content of corn the results compare more nearly with those obtained with *Puccinia coronata* on oats. It is very evident from Experiments 12–20 that the rust itself does not have any direct relation to light, for infection took place and the rust developed to spore formation in the dark. The same is also shown much better in experiments where carbohydrates were supplied to the host in the dark.

The relation of the rust to the carbohydrate supply is further seen when the host is deprived of its carbon dioxide supply (Experiments 22 and 23). For when the host is prevented from manufacturing carbohydrates by surrounding it with a carbon-dioxide-free atmosphere, there is no development of the rust.

A comparison of the infection of corn when supplied with carbohydrates alone and when supplied with carbohydrates plus Knop's nutrient shows varying results. In spite of the varying results, the experiments clearly show that in all cases there is a much greater infection when carbohydrates are supplied than when there are no carbohydrates present. It is probable that with the slow growth of the host the carbohydrates taken up by the host united with the nitrogen compounds formed in the metabolism of the host and formed proteins for the use of both host and fungus and so the rust did not feel the loss of the mineral elements to a very great extent.

It appears from experiments in which the host was deprived of its soluble carbohydrates that the rust was not able to live upon the host even though the host was alive and consequently did not use the protoplasm of the host, at least directly, as food. Marshall Ward (1904), especially, among the workers upon the rusts has noticed that the protoplasm of the host does not appear to be affected by the rust. In his Croonian lecture (1890), he points out that the relations of the rusts to their hosts are very different from those of a facultative parasite such as *Botrytis*. The rusts he considers as merely tapping the food supply of the host, establishing a relation which approaches symbiosis.

The results obtained agree with Ward's view. The development of the rust upon seedlings or cut leaves of the host furnished with carbohydrates, and the non-development except in a few cases, upon hosts furnished only with distilled water or mineral nutrient indicate that the rust is dependent upon the food supply of the host and does not live upon its protoplasm. The healthy development of host tissue in the infected regions compared with the surrounding dying tissue also shows that not only does the rust not live upon the protoplasm of its host but it even stimulates it to greater activity.

It is possible that the lack of carbohydrates might produce products in the host which are toxic to the rust. Thus amino acids and other products of metabolism might form which in the presence of carbohydrates would unite with them to form proteins again. These products might then inhibit the development of the rust. Experiments 38–40 indicate, however, that such is not the case. In these experiments where cut pieces of leaf were floated upon water and mineral nutrient such products would have had plenty of chance to diffuse out of the leaf. Yet on those solutions deprived of carbohydrates, there was no infection.

Thus the food material for which the rust depends upon the host appears to be the sugars or some of the compounds which they enter into during the formation of proteins, or what is more likely both. Such being the case it should be feasible to grow the rusts saprophytically. So far as I know only two attempts have been made to do this. These have been made by Carleton (1903) who obtained negative results and Ray (1901, 1903) who reports having grown several in culture. As has been pointed out above Ray's results are open to criticism due to the incomplete account of his methods and material.

In the case of *Puccinia Sorghi*, I have not been able to find that various nutrient media had any appreciable effect towards developing a mycelium. Various carbohydrates and organic nitrogenous products with and without mineral salts and at different concentrations showed an effect only upon spore germination and the length of the germ tube (Experiments 41–43). Sterilized pieces of leaf upon solutions such as were used successfully with living pieces of leaf, sterile decoctions of the host and water extracts of the host gave no better results. In all cases, the germ-tube produced by the spore lived only a few days.

Since *Puccinia Sorghi* does not appear to be able to use the sugars directly, but must have them supplied to the host for their development, it appears that it is not the stable carbohydrates or proteins which are to be considered as essential for the metabolism of the rusts. Rather, it is probable that the rust is dependent upon some transitory products in the formation of these substances, as Fromme (1913) has suggested, or it may be that the rust is able to utilize such compounds only in their nascent state, so to speak, when these complex organic compounds are not in a condition of equilibrium. Even among the saprophytic fungi, we have many which prefer certain stereoisomers and it would not be at all surprising to have in the rusts a group of fungi which needs certain isomers for their development. It is in some such explanation very likely that the obligate parasitism of the rusts is to be sought.

V. SUMMARY

1. The optimum temperature for the development of *Puccinia coronata* and *Puccinia Sorghi* is situated at about 20° C. and the maximum for *Puccinia Sorghi* is about 30° C.

2. While *Puccinia Sorghi* is not prevented from developing upon the host under conditions of dry air and soil, moist soil and a humid

atmosphere favor the development of the rust and increase the number of spores formed.

3. *Puccinia coronata* and *Puccinia Sorghi* do not appear to injure the cells of the infected area. The injury produced appears first in the areas surrounding the infected regions. This is probably due to a starvation brought about by a withdrawal of food from them by the infected areas.

4. A starvation of the host of various elements does not bring about immunity from the rust, but reduces the quantity of the rust produced.

5. Light is not necessary for the development of *Puccinia coronata* and *Puccinia Sorghi* when the host is able to obtain a good food supply.

6. When deprived of carbohydrates, light is necessary for the development of *Puccinia coronata* and *Puccinia Sorghi* in that it is necessary for the formation of carbohydrates by the host.

7. When deprived of carbon dioxide, the development of *Puccinia Sorghi* is stopped due to a lack of carbohydrates in the host.

8. Pure cultures of *Puccinia Sorghi* can be maintained upon both sterile seedlings and upon pieces of *Zea Mays* leaf floated upon carbohydrate solutions.

9. *Puccinia Sorghi* develops and forms spores upon seedlings or cut pieces of corn leaf when these are supplied with starch, cane sugar, dextrose, maltose, and dextrin in the dark.

10. When either seedlings or pieces of corn leaf are exhausted of carbohydrates and supplied only with mineral nutrient or water, *Puccinia Sorghi* is not able to develop in the dark.

11. *Puccinia Sorghi* is not able to use maltose, dextrose, cane sugar, asparagine, leucine, peptone with and without mineral salts, or decoctions of the host when supplied to it directly.

12. The obligate parasitism of the rusts is probably explained by their requirement of some transitory or nascent organic products related to the carbohydrates which they obtain in the living host.

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EXPLANATION OF PLATES IV AND V

Plate IV

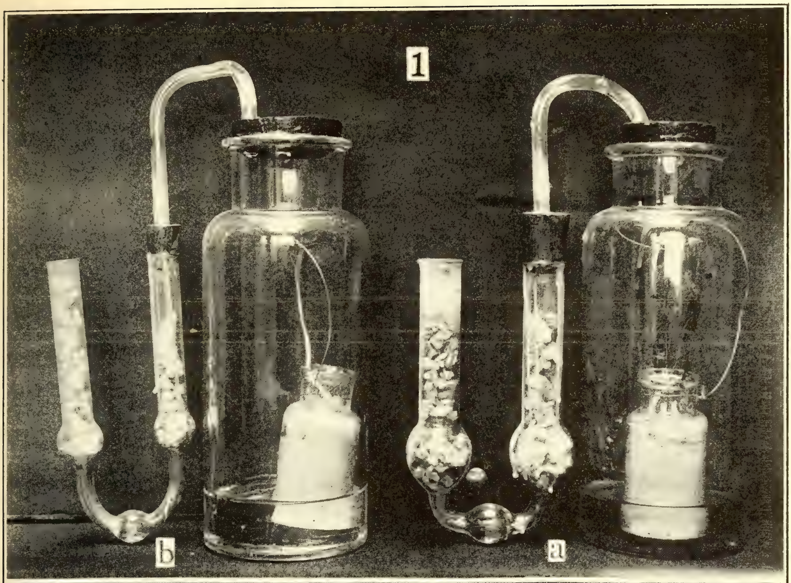
Fig. 1. Development of *Puccinia Sorghi* in carbon dioxide free atmosphere. *a.* Plant in carbon dioxide free atmosphere (uninfected). *b.* The check (infected).

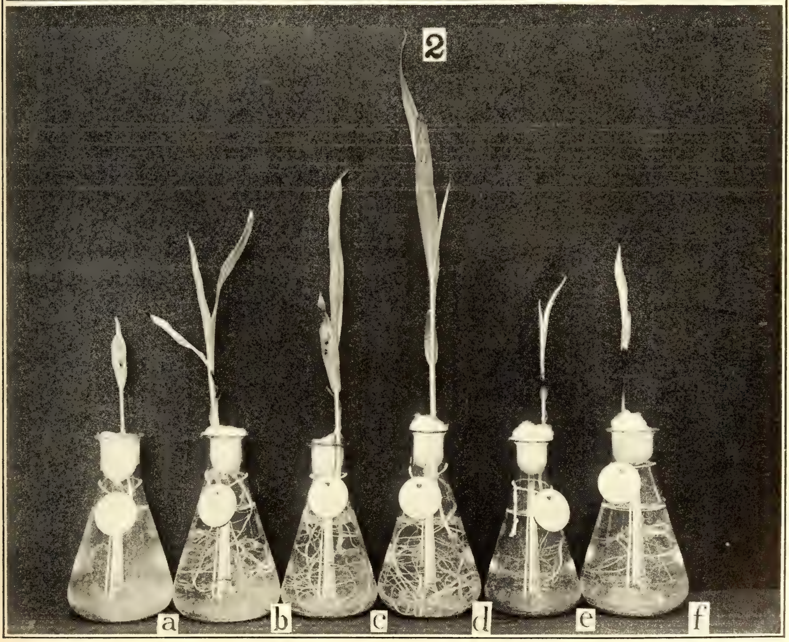
Fig. 2. Development of *Puccinia Sorghi* upon plants supplied with carbohydrates. *a.* Cane sugar 12 percent (infected). *b.* Cane sugar 6 percent (infected). *c.* Cane sugar 3 percent (infected). *d.* Cane sugar 3 percent plus Knop's mineral nutrient (uninfected). *e.* Knop's mineral nutrient (uninfected). *f.* Distilled water (uninfected).

Plate V

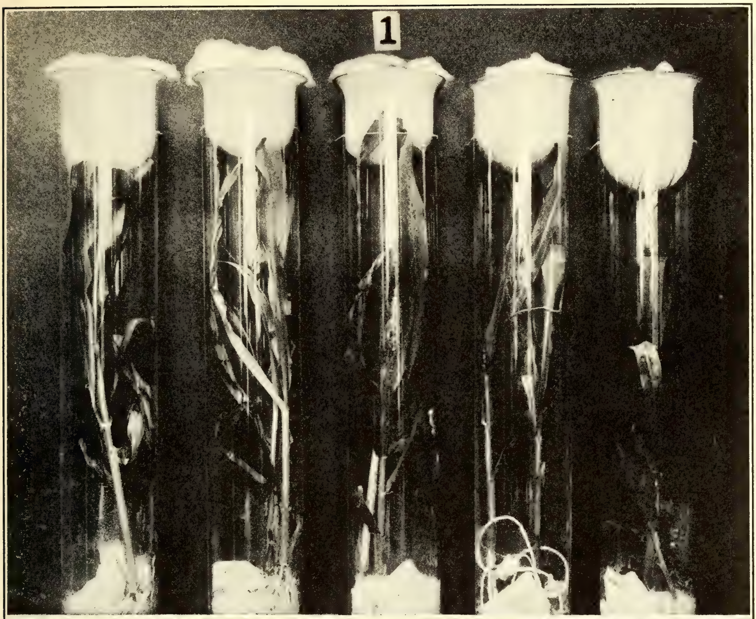
Fig. 1. Pure culture of *Puccinia Sorghi* and its host.

Fig. 2. Pure culture of *Puccinia Sorghi* upon cut pieces of corn leaf floated upon carbohydrate solution (looking down upon the capsules containing the leaves).





MAINS: RELATION OF RUSTS TO PHYSIOLOGY OF THEIR HOSTS



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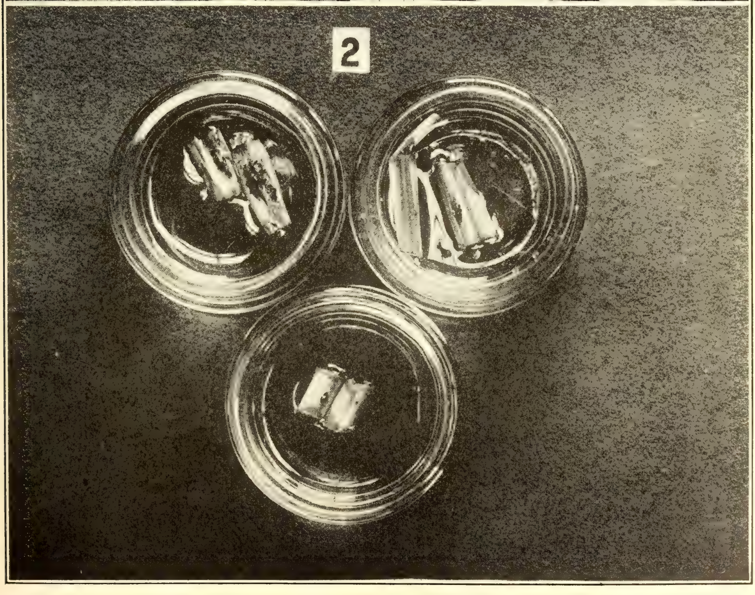

2

MAINS: RELATION OF RUSTS TO PHYSIOLOGY OF THEIR HOSTS

THE DEVELOPMENT OF SOME SPECIES OF AGARICS

A. W. Blizzard

The species of Agaricaceae whose lamellae are endogenous in their origin have in recent years been given considerable attention. The structural variation of a number of forms has been observed and studied. As a result a number of interesting morphological features have been explained and their development demonstrated. But very little attention has been given to those species whose lamellae are exogenous in their origin. It is important that the morphological characters of both forms be studied from their origin, beginning with the young, undifferentiated basidiocarp and tracing their development to the mature fruit body.

Hoffmann was the first to give serious attention to the development of Agaricaceae. In 1856 (13) he described *Panus torulosus*, showing that the lamellae are exogenous in their origin. He observed the hyphae of the young basidiocarp to diverge at the apical end, and noted the subsequent development of the pileus.

In 1860 (14) and 1861 (15) he followed this work with a description of a number of forms, the greater portion having an exogenous origin of the lamellae. He observed the early stage of the palisade layer, preceding the origin of the lamellae, to be level.

DeBary in 1859 (6) and 1866 (7) described *Nyctalis asterophora*, *N. parasitica*, *Collybia dryophila*, and others. In the main he agreed with Hoffmann's observations, with the exception of the condition of the palisade layer just previous to the development of the lamellae. DeBary stated that this layer was folded from the first. He later (8, 9) agreed with Hoffmann.

Fayod (11) in 1889 described a number of forms. He concludes that the pileus primordium is endogenous in origin in all Agaricaceae.

Since Fayod's work there are no published studies on the development of exogenous forms. In view of these facts, it appeared to me that it would be interesting to study the development of certain species whose lamellae are exogenous in origin.

Material.—Basidiocarps of three species in all stages of development were collected, August and September, 1915, near Seventh Lake, Adirondacks, N. Y. One species, *Clitocybe adirondackensis*, was collected late in September of the same year in Coy Glen, near Ithaca, N. Y.

Omphalia chrysophylla was found growing on a coniferous log which was not very far along in decay. The season being unusually wet, quite sufficient moisture was present, which produced a very luxuriant growth. The cells and structure of this species stand out with unusual clearness, due, very likely, to the firmness of the cell walls.

Clitocybe adirondackensis was found growing gregariously among leaves on a steep hillside under coniferous trees. The whitish mycelium growing through and among the decaying leaves, spread over a space equal to about three square feet.

Clitocybe cerussata was growing in leaf-mold under coniferous trees. The mycelium was very abundant and spread in all directions.

Clitopilus noveboracensis was growing in leaf-mold in a mixed forest. Its habit is gregarious. The white mycelium in forms of hyphal strands permeated the substratum, covering an area equal to about four feet square.

The basidiocarps were fixed immediately in Carnoy's fluid, cleared in cedar oil, embedded and sectioned in paraffine.

Omphalia chrysophylla Fries

Basidiocarp Primordium.—The youngest stages obtained of this species were small, elongate bodies, averaging 50μ in diameter and 780μ in length (Fig. 1). They are larger at the base and gradually taper to a blunt point at the apex. At this stage of development they consist of a homogeneous weft of slender threads, measuring 3.5μ in diameter. Their general direction is parallel with the axis of the young fruit body. The number of hyphae is increased by branching which takes place more abundantly toward the base in the young basidiocarp. The more peripheral hyphae end on the surface at varying distances from the tip, while central ones converge at the apex (Fig. 18), thus giving to the young fruit body its slender form.

Stipe Primordium.—The stipe primordium develops by continued growth of the hyphae that compose the undifferentiated basidiocarp and is not differentiated as such, until the origin of the pileus primordium; even then there is no definite line separating it from the pileus (Fig. 3).

In Figure 2, a slightly older stage, the hyphae at the base are more loosely interwoven. This results largely from the increase in the length of the threads and contributes to the enlarging of the base of the young basidiocarp. As the plant develops the hyphal cells increase in size until in the stage represented by Figure 3, they average 4 to 5μ in diameter and 10 to 20μ in length. The hyphae near the surface are more closely interwoven.

Pileus Primordium.—In Figure 19 (a higher magnification of a portion of the apex of Fig. 2) the apex has increased in breadth by a slight spreading of the hyphae and by interstitial growth of its elements. There is no differentiation in staining, but the whole structure has the appearance of very active growth. In a little later stage, Figure 20, the growth direction of these apical threads is out and upward, with a slight tendency, of the lateral ones, to epinasty. At the same time profuse branching takes place which supplies new elements that interlace and ramify among the older ones. Thus, in a longitudinal section, a weft of closely interwoven hyphae is presented, except at the periphery where the terete ends of hyphae, advancing in growth, project (Figs. 20 and 21). This divergent growth of the hyphae at the apex of the young fruit body marks the origin of the pileus and differentiates it from the stipe primordium. The origin of the pileus primordium corresponds very closely to that described by DeBary in *Nyctalis asterophora*, *N. parasitica* (6), and *Collybia dryophila* (8, 9).

Further Differentiation of the Pileus.—By continued growth of the primordial elements the pileus is delimited from the stipe in the following manner: The central hyphae continue to grow toward the surface, some curving outward more than others. This growth is accompanied with vigorous branching and interlacing of hyphae, which add new elements. In this way the inner structure of the pileus is formed (Figs. 3–6). At the same time the hyphae, by branching and growing in a radial direction, accompanied by strong epinasty, curve downward and form the margin of the young pileus (Fig. 22). By this radial and downward development of the margin of the pileus an annular groove, Figure 6, is formed on the surface of which is the hymenophore primordium. Figures 3–6 show the gradual development from the primordial condition of the pileus to that stage in which it is well differentiated from the stipe.

The pileus continues to increase in thickness and diameter in a manner similar to that described above and gradually changes to a broadly convex form with an incurved margin (Figs. 7–10).

Hymenophore Primordium.—Simultaneously with the formation of the annular groove by epinastic growth of the marginal hyphae, the hymenophore primordium is differentiated by the rich content in protoplasm of the hyphae forming the external annular zone in the furrow. They are crowded, and stained deeply as shown in longitudinal section (Fig. 6).

The annular region is composed of more or less blunt and cylindrical ends of numerous hyphal branches which have their origin both in the stipe and pileus elements. Their growth direction is obliquely out and downward. The oldest are on the stipe and by centrifugal development new elements are added to this area near the margin of the pileus which continues to curve down over this surface. Figure 22 is a median longitudinal section of the fruit body at this stage of development and shows this structure in detail.

At first this annular primordial layer curves out and upward at an angle of about 45°. As the pileus expands and becomes more convex, new primordial elements are introduced by branching and interstitial growth in centrifugal succession as above described. This causes it to curve in the form of an arch (Figs. 7–8).

Since the development is centrifugal it must be borne in mind that, at the time this layer is in the primordial stage at the margin of the pileus, near the stipe it will be further differentiated.

Palisade Layer; Origin and Development of the Lamellae.—The hyphae of the hymenophore primordium branch in a digitate manner. By this branching new elements are interpolated in the spaces between the older hyphae. This process continues gradually until a compact layer of short hyphae is formed. Simultaneously with this the cells enlarge, especially the terminal ones, and the surface smooths up into an even, compact layer (Fig. 23). This is the palisade layer and precedes the origin and development of the lamellae, as has been described for a number of endogenous forms. The differentiation of the palisade layer appears first near the stipe and progresses centrifugally toward the margin of the pileus, as did the development of the hymenophore primordium. As the cellular elements of the palisade layer increase in size, a great pressure is produced within this structure. This pressure is released to some extent by the palisade layer being thrown into equally spaced, radial folds beginning near the stipe (Fig. 12).

Simultaneously with this, subadjacent hyphae along radial areas corresponding with the gill areas, by elongation, push their way downward and govern the origin of the gill salients. Figures 24 and 25 show this feature in excellent detail. These down-growing salients of the level palisade layer are the first evidence of the appearance of the lamellae. Continued growth of these salients produces the lamellae, as observed in species of Agaricus (1, 2, 4), Coprinus (5), Cortinarius (10), etc.

The subhymenial hyphae are branched in a corymbose manner, Figure 27, and supply new elements within the hymenophore layer. Figure 28 is a higher magnification of a portion of Figure 27 and shows in detail the corymbose branching.

Growth in width of the lamellae occurs by the further elongation of the tramal hyphae which branch as above described. These new elements are interpolated between the older hyphae at the edge of the gill.

The development of the lamellae is centrifugal as are the structures preceding their origin. Therefore the oldest portions of the lamellae are nearest the stipe and proceed in a radial direction to the margin. Since the margin of the pileus is involute, a tangential section through that portion of the fruit body parallel with the axis of the stipe will present an appearance as represented by Figure 16. Below, it shows a portion of the inrolled pileus edge. This relation of the gills to the involute margin of the pileus has been adequately described by Atkinson for *Agaricus rodmani* (4).

Structure of Stipe and Pileus.—As the basidiocarp grows the stipe becomes even in diameter. This results largely from the elongation of the peripheral hyphae and more abundant branching in the upper portion of the stipe, together with the enlargement of the cellular elements. The process is a gradual one, as Figures 3–9 show.

The pileus at the same time increases in all its dimensions and becomes more expanded (Fig. 10). The general direction of its elements is horizontal and radial (Fig. 29). Those on the surface are more closely interwoven, and this serves to produce a smooth surface. Figure 30 is a high magnification of a portion of the pileus which shows this structure very clearly. The hyphal cells have very firm walls and are exceedingly large, measuring 7 to 8μ in diameter.

Clitocybe adirondackensis Peck

Basidiocarp Primordium.—The undifferentiated basidiocarps of this species are long, slender bodies, tapering toward the apex. They are usually curved or bent in various directions. Those studied measure 60μ in diameter and 1 mm. to 2 mm. in length. The hyphae are very slender, wavy threads, averaging 1.5μ in diameter. They run in a longitudinal direction (Figs. 31 and 41). The central hyphae extend to the apex where they converge into a blunt point. The peripheral ones end in such a manner as to form a slanting surface from the base to the apex. There is no differentiation at this time, the whole fruit body staining homogeneously.

Pileus Primordium.—When the young primordium of the fruit body reaches a diameter of about 300μ the lateral threads at the apical end begin to spread laterally (Figs. 32 and 42). The central hyphae continue their growth upward. The interhyphal spaces are filled by new elements which are produced by branching of the older hyphae. This structure is the pileus primordium, and, as in *Omphalia chrysophylla*, the divergence of the threads indicates the differentiation of the pileus fundament at the apex.

Further differentiation of the pileus is the result of continued growth of this primordial tissue. Around the upper lateral surface of the stipe primordium and on the under side of the young pileus, the ends of the diverging hyphae stain deeply and mark the origin of the hymenophore fundament (Fig. 33). The central apical hyphae continue their growth upward and by profuse branching add materially to the thickness of the pileus, while the intermediate elements bend gently outward.

By continued branching and interstitial growth of its elements the pileus increases in diameter. At the same time the central hyphae, as compared with those of the periphery, elongate less rapidly. Thus the intermediate and peripheral threads, growing upward and outward at an oblique angle of about 45°, cause the pileus to become plane on its upper surface (Fig. 35). The marginal hyphae at the same time continue to curve abruptly downward. In this way a shallow and very narrow annular groove is formed.

In later stages in the under portion of the pileus next the stem, hyponasty replaces epinasty. The form of the pileus consequently changes from plane to umbilicate, and then to infundibuliform, while epinasty continues to have its influence on the thin younger margin which is incurved or involute.

Structure of the Stipe.—At the appearance of the pileus fundament, the stipe primordium is clearly differentiated as a definite region. At this stage the apparently homogeneous structure of the stipe primordium is changed by the loosening up of its texture. There are strands of tissue running longitudinally through the stipe. These strands stain very deeply, causing them to stand out conspicuously. They intertwine with others in anastomosing fashion, thus forming intervening hyphal spaces.

The stipe elongates by the lengthening of the cells. These elements, in the stage of development represented by Figure 40, measure 3–30μ in length. The increase in width is the result of branching and interstitial growth of the hyphae, and also by the increase in diameter of the cells themselves, which average 3.5 to 4μ. In more mature plants increased thickness is chiefly the result of the latter, as Hoffmann (14) on page 394 suggested.

Hymenophore Primordium.—The organization of the hymenophore primordium occurs simultaneously with that of the origin of the pileus margin. Like it, too, the development is centrifugal. The first differentiation of this tissue is in the angle between the pileus margin and stipe and on the upper surface of the stem. Because of the active increase in its elements and their richness in protoplasm the young hymenophore primordium takes a dense stain.

As the pileus increases in width, its marginal hyphae add to this annular zone so that its surface is increased radially and upward (Fig. 43). Its elements multiply by intercalary growth and present a frazzled appearance, as observed in *Coprinus comatus* (5), *Agaricus rodmani* (4), and some other plants.

Palisade Layer, Origin and Development of the Lamellae.—By continued branching of the hyphae, the zone of primordial elements organize a definite layer of parallel threads which becomes more or less even on the surface since the ends of the hyphae reach the same level. This results in forming a compact layer of parallel threads perpendicular to the surface. Figure 44, is a transection through the upper part of the stipe and shows a portion of this structure immediately beneath the curved pileus margin. The hyphal elements of this layer are slender, cylindrical, septate threads, 4.5–6μ in diameter and 35μ in length. The terminal cell is longer than the others of the same thread and slightly larger, which tends to give a clavate appearance to the threads. The cells are rich in protoplasm and present an appearance of active growth.

Hymenophore Primordium.—The lamellae make their first appearance as folds of the level palisade layer. These folds are the rudiments of the lamellae themselves. They appear on the surface at or near the apex of the stipe, Figure 36, and by progressive growth extend out and upward on what is the morphological underside of the pileus. By downward growth of hyphae subadjacent to these folds, the trama of the lamellae is formed (Figs. 45–46). These tramal threads are differentiated from the other elements of the hymenophore by the fact that they do not stain so deeply. These threads branch and furnish new elements by which the lamellae grow in thickness and at the same time by apical and intercalary growth the lamellae increase in width (Fig. 47).

The tissue of the pileus and stipe subadjacent to the hymenophore is peculiar because of extraordinary large interhyphal spaces, due to the extension exerted by the pressure from interstitial growth and enlargement of the elements of the hymenophore.

The lamellae develop in length in a radial centrifugal direction, following that of the palisade layer. They are decurrent from the beginning, since the hymenophore has formed around the upper lateral surface of the stipe (Figs. 36–40). At the base of the older ones, other lamellae sometimes branch off, developing in a manner described for the primary gills (Fig. 37). These form the forked lamellae sometimes present in this species. Secondary lamellae also arise between the diverging primary gills, filling the spaces between them.

Clitocybe cerussata Fries

Basidiocarp and Stipe Primordia.—The youngest basidiocarps of this species which were collected measure .5 mm. in diameter and 2 mm. in length (Fig. 70). They are composed of slender interlacing hyphae, measuring 3μ in diameter, which form a close interwoven tissue. Their general direction is longitudinal, converging at the apex (Fig. 83). This homogeneous structure is the primordium of basidiocarp and stipe.

By continued growth of this primordial tissue the stipe fundament is finally differentiated by the formation of the pileus primordium which is marked off by the divergence of the apical hyphae. As the stipe becomes older the hyphae are more loosely interwoven (Fig. 7). Its further growth is provided for by means of branching and elongation of its elements.

Pileus Primordium.—At the time the stipe fundament is delimited from that of the pileus, the apical hyphae grow upward and spread out in all directions. In this feature it is similar to that of *Clitopilus noveboracensis*, which is described below. The hyphal elements are long, slender and terete. This is the pileus primordium (Fig. 84).

By continued radial and diverging growth of its elements the pileus fundament increases in size (Figs. 72, 77). This gives rise to a hemispherical body which is delimited from the stipe by the annular groove (Figs. 72–74). Further epinastic growth causes the margin to curve inward toward the stipe (Fig. 76). At this time the plant has assumed a beautiful and symmetrical form. In Figure 77, the pileus has enlarged and the margin has become so strongly involute that the edge turns upward against the gills. The hyphae do not grow out from the margin of the pileus nearly so strongly as in *Clitopilus noveboracensis*.

Hymenophore Primordium.—The hymenophore fundament is differentiated in the annular groove between the pileus and stipe and stains deeply. This area develops in a radial manner, following the centrifugal growth of the pileus, characteristic of the Agaricaceae. This area consists of short hyphae perpendicular to the surface of the annular groove. It becomes more dense by interpolation of new elements which are formed by digitate branching of the primordial hyphae.

Palisade Layer; Origin and Development of Primary Lamellae.—As the hymenophore becomes more compact by intercalary growth, the cells themselves increase in size. The end of the hyphae reach the same level and form an even palisade layer, as shown in Figure 78. A higher magnification is shown in Figure 85. The hyphal elements that compose this layer are longer than those of the other species described in this paper, and are comparatively slender.

As the elements increase in number and size, the resulting pressure is partly relieved by the level palisade layer bulging out into radial fold-like ridges. These ridges are the gill fundaments. In this species, as in *Clitopilus noveboracensis*, they occur first on the stipe very near the angle between the latter and the lower surface of the pileus (Fig. 79). Later the gill salients of the primary lamellae appear on the under surface of the pileus as shown in Figure 80. In this figure, on each side of the salients, a portion of the palisade layer is shown. Since these structures develop centrifugally the first differentiation occurs on or near the stipe. Consequently in a tangential section the portion to the right or left would be cut obliquely and show tissue nearer the margin than that in the center of the section. Thus, the palisade layer represents a younger portion of the hymenophore, in which salients have not as yet made their appearance.

The development of the lamellae in width is as has been described for the previous species. The subadjacent hyphae by elongation, aid in the extension of the salients in width or keep pace with their growth. New elements are also added by intercalary growth to the palisade layer. Figures 85–89 show in detail the development of a gill from the palisade stage of the hymenophore through the first evidence of a gill salient to a well-formed lamella.

Origin of Secondary Lamellae.—As was described for the previous species, the salients of the secondary gills appear between the primary lamellae on the under surface of the pileus. Those that appear first occur near the stem (Fig. 81). Their development is exactly as described for the primary gills. They serve to occupy the spaces produced by the divergence of the primary gills as they proceed from the stipe.

Structure of Pileus and Stipe.—The more mature pileus is expanded and the hyphae arrange themselves in a radial horizontal direction. The trama is composed of hyphal threads that ramify and interlace among themselves. The stipe increases in width by branching and interstitial growth of the hyphae. In the more mature pileus and stem, growth is chiefly by the increase in size of the cellular elements.

Clitopilus noveboracensis Peck

Basidiocarp and Stipe Primordia.—The fruit bodies representing the primordial stage of the basidiocarp become comparatively large, .6 mm. in width and 2 mm. in length, before differentiation of the pileus occurs. They are elongate bodies which taper gradually to a point at the apex (Fig. 48). The young basidiocarp presents a closely interwoven structure composed of slender hyphae averaging about 3μ in diameter at the base; toward the apex they are not so stout. The general direction of the hyphae is parallel with the direction of the growth of the fruit body (Fig. 64). The whole extent of the apical end and a portion of the peripheral hyphae stain deeply, which indicates an area of active growth.

This fundamental tissue is soon differentiated by growth direction of the apical threads as stipe primordium. At this time the inner structure of the stipe fundament is a woof of slender homogeneous hyphae, while some of the hyphal threads near the surface of the stipe, growing more rapidly than the other elements, extend outward and form a loose floccose layer. Figure 49, a median longitudinal section, shows this layer as a narrow zone which stains more deeply. This structure is composed of the dead ends of those hyphae which extend beyond the immediate surface of the stipe and is very ephemeral.

Pileus Primordium.—The origin and development of the pileus fundament agrees with the preceding species. The elements extend outward in all directions with a slight tendency to epinasty. In this species the fundament consists of a peripheral zone of long radiating hyphae and a dark staining central portion. The hyphae of both the loose and the more dense regions have the same origin; *i. e.*, the elements of both regions are the result of radial diverging growth from the stipe fundament.

Further development of the pileus is by the continued radial growth of the hyphae. At the margin by epinastic growth the hyphae curve downward, forming the annular groove (Figs. 50, 51). In the stages represented by Figures 52–54, the hyphae branch profusely and are organized in a very compact structure, except a very thin, loose surface zone. The pileus margin develops by centrifugal growth. On the surface of the annular groove in the angle between the pileus and stipe the hymenophore fundament is organized.

In a later stage of development, represented by Figure 55, the pileus margin is so strongly involute that the edge is curved upward against the lamellae. The marginal hyphae span the intervening space between the pileus margin and the gills. At this stage of growth these hyphae function as a marginal veil, though this veil is very different in origin from the marginal veil of those species with endogenous origin of the hymenophore. They do not, at any stage of development which I have examined, interlace with the hyphae of the stipe, as Hartig suggested for *Armillaria mellea* (12). Such an interlacing might occur in case the margin curved down against the stipe below the hymenophore area. In all the specimens examined, however, the pileus margin curves up toward the hymenophore. Hyponastic growth of the older portion of the pileus begins soon, causing it to expand, thus lifting the margin up and outward far away from contact with the hymenophore. The growth of the pileus in thickness is primarily the result of the enlargement of its elements accompanied by branching and intertwining of the threads.

Hymenophore Primordium.—Soon after the origin of the pileus primordium, the ends of the peripheral hyphae which are perpendicular to the surface of the annular groove, are rich in protoplasm and stain deeply. This is the region of the hymenophore primordium. This region, as in *Omphalia chrysophylla* and *Clitocybe adirondackensis*, develops centrifugally and adds new elements by intercalary growth.

Palisade Layer.—By continued introduction of new elements, this layer becomes compact and the free ends reach the same level (Fig. 66). The increase in size of the cellular elements and the extending downward of the subadjacent hyphae produce regularly spaced, radial folds in the palisade layer (Figs. 57, 58). These folds are the salients of the primary lamellae and appear first on the stipe very near the angle between the latter and the under surface of the pileus. Thus, the gills are decurrent from their very first appearance.

At this period of development the hymenophore layer on the under surface of the pileus is in the level palisade stage, near the stipe (Fig. 56). It gradually grades off into the primordial condition at the margin. Therefore, since the gills follow the same centrifugal succession as did the structures preceding their origin, the salients continue their development toward the margin of the pileus. Thus, Figure 58 (a little later stage than Fig. 57) shows their first appearance on the under surface of the pileus.

Further growth in width of the lamellae is brought about by growth of the tramal hyphae in these folds. This growth aids in pushing the palisade layer outward at the edge of the salient. By corymbose branching new elements are introduced into the palisade layer by intercalary growth. The hyphae that grow down into the lamellae from the trama of the pileus form the trama of the gills. Figures 67–69 show a serial development of a gill from the origin of the salient to a lamella fairly well along in growth.

Origin and Development of Secondary Lamellae.—As the primary gills advance from the stipe to the margin of the pileus, they diverge from each other. In the spaces so produced on the under surface of the pileus near the stipe, the secondary lamellae arise. Figure 59, a transverse section through the stipe and pileus, shows the primary lamellae as "bars" extending between the pileus and the stipe. Between the "bars" on the morphological under surface of the pileus, down-growing salients of the secondary gills are shown. They develop and progress radially, as do the primary lamellae. Figure 61 is a slightly oblique transection through the margin of the pileus and upper portion of the stipe and shows the increase in number of lamellae on the pileus margin as compared to the number of primary gills on the stipe.

Further Growth of Pileus and Stipe.—The pileus elements in the more mature stage have in general a radial, horizontal direction. The trama of the pileus increases by branching and elongation of its elements. The size of the stipe increases likewise by branching and interstitial growth. The lengthening or elongation of the stem, as in the previous species studied, is the result of the extension in length. of the cellular elements.

SUMMARY

1. The young basidiocarp and stipe primordium consist of a homogeneous weft of slender, terete, interlacing hyphae. The general growth direction of the elements is parallel with the axis of the young fruit body. The hyphae converge at the apex. The cellular elements are comparatively short, cylindrical cells, rich in protoplasmic content.

2. The pileus primordium is differentiated by the divergence of the apical hyphae which grow upward and laterally. This divergence serves to mark the origin of the pileus and differentiates it from the stipe fundament.

Further differentiation of the pileus is the result of continued growth of the primordial tissue. By profuse branching and interstitial growth of the elements, an intricately interwoven tissue is produced. At the same time the lateral hyphae by epinastic growth bend downward, forming the annular groove.

3. The primordium of the hymenophore is organized simultaneously with the origin of the pileus margin. The first differentiation of this tissue is on the surface of the annular groove in the angle between the pileus and stipe. This annular layer progresses centrifugally.

4. By continual branching of the hyphae, the hymenophore primordium changes to a definite layer of parallel threads, perpendicular to

the surface. By the enlargement of the cells of these parallel hyphae, and the evening up of the hyphal elements, a level palisade layer is produced.

5. The primary lamellae originate as evenly spaced, radial folds of the level palisade layer. The first folds that appear are the rudiments of the primary gills. Their further development is produced by the elongation of the subadjacent hyphae of the pileus which push their way into the salients and form the trama of the gills. These tramal hyphae branch and furnish new elements by which the lamellae grow in thickness and at the same time by apical and intercalary growth increase in width.

6. The secondary lamellae arise as down growing salients of the palisade layer on the under surface of the pileus near the stipe between the primary gills. They develop as do the primary lamellae.

In conclusion I wish to express sincere thanks to Professor Geo. F. Atkinson under whose direction this study was undertaken for many helpful suggestions.

Department of Botany, College of Arts and Science,
Cornell University

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DESCRIPTION OF PLATES VI–XI

The following photomicrographs were made by the author with a Bausch and Lomb vertical camera and Zeiss lenses, and with a horizontal Zeiss camera.

PLATE VI

FIGS. 1–17. *Omphalia chrysophylla.*

FIGS. 1–10. Median longitudinal sections, showing the general habit and development of the basidiocarp. × 24.

FIG. 1. Basidiocarp primordium composed of a homogeneous weft of slender threads. The general direction of the hyphae is parallel with the axis of the young fruit body. × 24.

FIG. 2. The first differentiation of the basidiocarp is the flaring out of the hyphae at the apical end which is the region of the pileus primordium. The portion of the basidiocarp below this differentiation is the stipe primordium. × 24.

FIGS. 3–5. Sections showing the gradual development of the pileus. The hyphae grow up and outward with a slight tendency to epinasty. They also show the gradual growth that takes place in the stipe which causes it to become even. × 24.

FIG. 6. Shows the further development of the pileus and stipe. The former develops by a radial centrifugal growth. The marginal hyphae curve down and form an annular groove on whose surface is the hymenophore primordium. The latter structure is differentiated as a more dense staining area in the angle between the margin of the pileus and stipe. × 24.

FIG. 7. Represents a further development of these structures. The hymenophore primordium has differentiated into the palisade layer. × 24.

FIGS. 8–10. These sections show the continued development of the basidiocarp. The palisade layer has been replaced by the lamellae. The stipe and pileus has developed by means of branching of its elements and interstitial growth. × 24.

FIG. 11. A tangential section near the stipe, showing the palisade layer. × 24.

FIG. 12. A tangential section very near the stipe which shows the downgrowing salients of the palisade layer, the rudiments of the lamellae. × 24.

FIGS. 13–17. Tangential sections, showing the development of the lamellae from the first appearance of the salients to the well-formed lamellae. × 24.

FIG. 16. Is a tangential section through the margin of the pileus, showing the involute edge beneath the gills. × 24.

Plate VII

Figs. 18–30. *Omphalia chrysophylla.*

Fig. 18. A higher magnification of a portion of the apex of Fig. 1. It shows the converging of the apical hyphae and homogeneous nature of the whole structure. × 100.

Fig. 19. A higher magnification of a portion of the apex of Fig. 2. The threads are spreading apart slightly and have increased in size. This differentiation marks the region of the pileus and stipe primordia. × 500.

Figs. 20, 21. A higher magnification of a portion of the apices of Figs. 3–4, showing the further growth of the primordial hyphae of the pileus. They grow radially outward and by epinasty curve downward. × 300.

Fig. 22. A higher magnification of the pileus margin of Fig. 8, showing the annular groove on the surface of which is the hymenophore primordium. This primordium is composed of the ends of hyphae whose origin is in the pileus and stipe elements. They are rich in protoplasm and stain deeply. The hyphae of the pileus margin by strong epinasty curve down by which the annular groove is formed. × 230.

Fig. 23. A tangential section of the pileus near the stipe which shows in detail the structure of the palisade layer. This layer is formed by branching and interstitial growth of the primordial hyphae. As the cells themselves increase in size the layer becomes compact and even. × 300.

Fig. 24. A tangential section of the pileus showing the beginning of a gill salient. The pressure within the layer is relieved by this downward folding of the level palisade layer. At the same time subadjacent hyphae by elongating push down into this fold forming the trama of the gills. × 300.

Fig. 25. A tangential section showing a salient a little further developed. In this section the detail of the structure stands out so definitely that there can be no possible mistaking as to how the gill salient proceeds in developing. The tramal hyphae can be easily traced from the pileus elements above down into the palisade layer itself. In this way new elements are introduced in the periphery of the salient and also the trama of the gill is produced. × 300.

Figs. 26, 27. Are tangential sections showing further development of the gill salients. The tramal hyphae are evident and the corymbose branching, by which new elements are added to the palisade layer of the gill, is clearly shown. × 300.

Fig. 28. A higher magnification of a portion of the palisade layer of Fig. 27. The corymbose branching of the tramal hyphae and the intercalary growth of the elements are well shown. × 720.

Fig. 29. A median longitudinal section which shows the structure of the pileus and its relation to the palisade layer between the gills. The hyphae branching in a corymbose manner supply elements to the palisade layer. × 230.

Fig. 30. A high magnification of the edge of the pileus surface of Fig. 10. The very large and stout hyphae are well shown. The hyphae on the right side of the figure are interwoven and serve to produce a smooth surface. × 720.

PLATE VIII

FIGS. 31–47. *Clitocybe adirondackensis.*

FIG. 31. A median longitudinal section of a young basidiocarp. It represents the primordial stage at which time it is composed of a loose weft of wavy, slender, and homogeneous hyphae. × 32.

FIG. 32. A median longitudinal section which shows the pileus primordium at the apical end, differentiated from the stipe primordium. The apical threads are spreading and serve as an arbitrary line of demarcation between the areas of the two primordia. × 32.

FIG. 33. A median longitudinal section showing further development of the pileus and stipe. By continued growth of the central primordial hyphae umbonate pileus is produced. Between the margin of the pileus and stipe an area of densely staining hyphae is shown. This is the hymenophore primordium which develops centrifugally as the pileus continues to grow. × 32.

FIGS. 34, 35. A median longitudinal section of later stages. The pileus has increased in thickness but the expansion is comparatively little. Since the marginal hyphae elongate more rapidly than the central ones the pileus becomes plane. The hymenophore primordium develops at the same time, advancing toward the margin of the pileus and stains more deeply. × 32.

FIG. 36. An oblique transection through the upper portion of the stipe. The lower part of the figure shows a portion of the surface of the stipe beneath the hymenophore. To the left and above this region is a portion of the palisade layer. The remaining peripheral portion shows the folding of the palisade layer and the development of the gill salients. × 32.

FIG. 37. Cross-section of the extreme lower portion of the pileus, showing the general habit of the gills and manner of development. × 32.

FIG. 38. A tangential section through the pileus and near the stipe, showing the decurrency of the lamellae and also the incurving of the pileus margin. × 32.

FIG. 39. A tangential section midway between the margin of the pileus and stipe. It shows the thickness of the pileus and the nature and general direction of the gills. Some are connected at their base which is chiefly the result of branching. × 32.

FIG. 40. A median longitudinal section, showing the general habit of the plant. The pileus is plane and the gills extremely decurrent.

FIG. 41. A high magnification of a young basidiocarp, showing in longisection the structure of the primordial condition. A homogeneous weft of wavy, slender threads which converge at the apex. × 230.

FIG. 42. A median longitudinal section, showing the apical hyphae growing outward. This is the pileus primordium. × 300.

FIG. 43. A median longitudinal section, showing the hymenophore primordium. This is composed of the ends of hyphae which have their origin in the stipe and pileus elements. The margin of the pileus curves down over the surface. Thus, the marginal hyphae add new elements to the hymenophore regions, both of which develop centrifugally. × 300.

FIG. 44. A cross-section through the basidiocarp near the apex of the stem

immediately below the margin of the pileus. This shows the palisade layer which is formed by the gradual increase of primordial elements by intercalary growth. At the same time the cellular elements, which result in a compact layer. × 300.

FIG. 45. A cross-section similar to the above only of a slightly older stage. It shows the first appearance of gill salients, which are the outfolding of the palisade layer. Subadjacent hyphae grow into this fold, and by elongation force the salient outward, at the same time branching in a corymbose manner, new elements are added. × 300.

FIG. 46. A cross-section of the pileus which shows further growth of the gill salients. The hyphae that force their way down into the salients from the pileus elements do not stain so deeply and are easily distinguished. They form the trama of the lamellae. × 300.

FIG. 47. A cross-section of a little older stage than the preceding figure. This shows the apical development of the gill by which the lamellae increase in thickness.

PLATE IX.

FIGS. 48–63. *Clitopilus noveboracensis.*

FIG. 48. A median longitudinal section, showing the homogeneous weft of slender, interwoven hyphae. The peripheral hyphae end at varying distances from the tip, so that the surface slants gradually from the base to the apex. × 20.

FIG. 49. A median longitudinal section, showing the flaring of the hyphae at the apex which serves as a line of demarcation between the pileus and stipe primordia. On the surface of the stipe primordium is a very narrow zone of tangled hyphae which stain more deeply. This is composed of the ends of hyphae which project farther than those that compose the weft. × 20.

FIGS. 50–54. Median longitudinal sections, showing older stages of development. The marginal hyphae by epinastic growth turn downward, forming the annular groove on whose surface is the hymenophore primordium.

FIG. 55. A median longitudinal section of a more mature plant, showing its general habit. The margin turns in and upward towards the gills. The marginal hyphae extend outward as a loose weft and span the space between the pileus margin and gills. At this stage it has the function of a marginal veil. × 13.

FIG. 56. A tangential section through the pileus near the stipe, showing the palisade layer. × 20.

FIG. 57. An oblique transection through the margin of the pileus and upper part of the stipe. The cavity within represents the annular groove. On the surface of the stipe the palisade layer has been thrown into folds. These folds are the gill salients. Thus the origin of the primary gills is on the stipe. × 20.

FIG. 58. A tangential section of a young pileus, showing the origin of the primary gills as they extend from the stipe on the lower surface of the pileus toward the pileus margin. × 20.

FIG. 59. A slightly oblique transverse section through the pileus margin and upper part of the stipe, showing the origin of the secondary lamellae between the primary gills. The primary gills appear as "bars," connecting the pileus and stipe. × 20.

FIG. 60. A tangential section through the pileus, showing the decurrency of the gills; also the incurving of the pileus margin. × 20.

FIG. 61. A slightly oblique cross-section through the pileus margin and stipe, showing: (1) primary gills on the stipe; (2) the upper left hand portion of the figure shows two "bars" of the primary gills; (3) a secondary gill between the "bars"; (4) primary and secondary gills on the pileus margin. On the right lower hand portion of this figure is shown a part of the involuted pileus margin. × 20.

FIG. 62. A tangential section through the involuted margin of the pileus, showing the relation of the involuted margin to the gills. × 20.

FIG. 63. A tangential section through the margin of the pileus just within the limit of the involuted margin. It shows very clearly how the marginal hyphae have been pushed against the gills. × 20.

PLATE X

FIGS. 64–69. *Clitopilus noveboracensis.*

FIG. 64. A higher magnification of the apex of Fig. 48, showing the converging of the hyphae. × 200.

FIG. 65. A higher magnification of a portion of the pileus primordium, showing the spreading of the hyphae at the apex. × 200.

FIG. 66. A higher magnification of the palisade layer. Its elements are increased by intercalary growth. This, together with the increase in size of the hyphae, produces the compact palisade. × 300.

FIG. 67. A cross-section through the stipe, showing the first fold in the palisade layer. It is an outward growing salient, the rudiment of a lamella. × 300.

FIGS. 68, 69. Transverse sections through the pileus margin and stipe, showing further development of the gill salient. The apical growth of the gill is well shown by which it increases in width. The tramal hyphae can be definitely made out. × 300.

FIGS. 70–74. *Clitocybe cerussata.*

FIG. 70. A median longitudinal section of a young basidiocarp, showing the basidiocarp primordium. It is a homogeneous weft of slender, interlacing hyphae, whose general direction is longitudinal and converge at the apex. × 20.

FIG. 71. A median longitudinal section, showing the diverging hyphae at the apex. This serves to separate the pileus and stipe primordia. × 20.

FIGS. 72–74. Median longitudinal sections of older stages, showing the centrifugal development of the pileus. The stipe at the same time increases in thickness. The hymenophore is differentiated in the surface of the annular groove. × 20.

FIGS. 75, 76. Median longitudinal sections, showing further growth of the pileus. The margin curves down and toward the stipe. × 20.

FIG. 76. A median longitudinal section of a more mature plant, showing its general habit. The strongly incurved margin is well shown. × 20.

PLATE XI

FIGS. 78–89. *Clitocybe cerussata.*

FIG. 78. A tangential section of the pileus, showing the palisade layer near the margin. × 20.

FIG. 79. A slightly oblique transection through the pileus margin and stipe, showing the origin of the lamellae as folds in the palisade layer on the upper portion of the stipe. × 20.

FIG. 80. A tangential section through the pileus, showing the origin of the primary gills on its under surface. On either side of the down-growing salients a portion of the palisade layer is shown. × 20.

FIG. 81. Transection of the pileus and stipe, showing the origin of the secondary lamellae between the primary gills which show as "bars." × 20.

FIG. 82. Tangential section of the pileus, showing further development of the gill salients. The tissue below the salients is a portion of the involuted pileus margin. × 20.

FIG. 83. A higher magnification of the apex shown in Fig. 20. This shows the converging of the apical threads and the general homogeneous structure. × 100.

FIG. 84. A longitudinal section of the pileus primordium, showing the general growth direction of the hyphae. × 300.

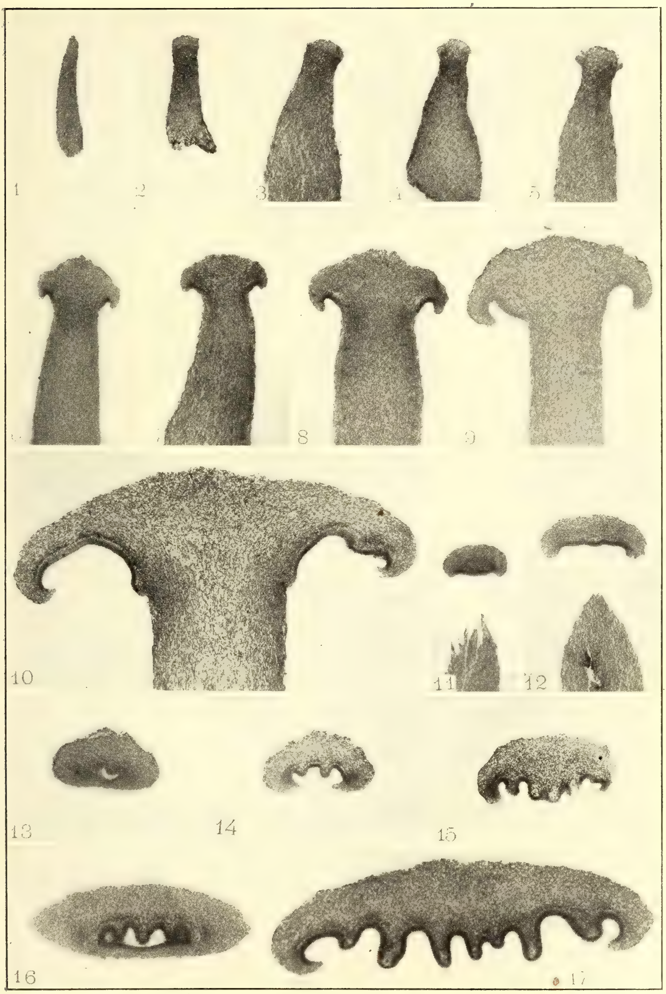
FIG. 85. Transection of the stipe, showing the palisade layer. × 300.

FIG. 86. A transverse section of the stipe, showing the first evidence of a fold in the palisade layer. × 300.

FIG. 87. Further development of a salient represented by Fig. 86. It shows the apical growth by which the elements of the palisade layer are increased by elongation of the hyphae from the trama of the pileus. × 300.

FIG. 88. Represents a little older stage than the preceding one. The tramal hyphae are shown growing down into the gill and branching; thus new elements are supplied to the palisade layer. × 300.

FIG. 89. A slightly older stage, showing further development of the gill. × 300.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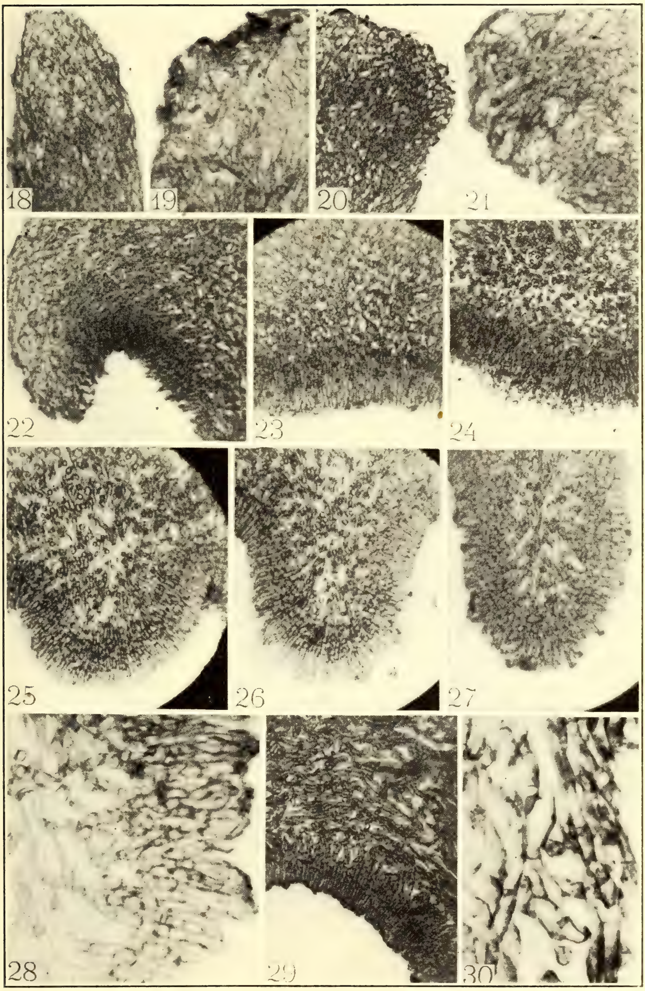
BLIZZARD: OMPHALIA CHRYSOPHYLLA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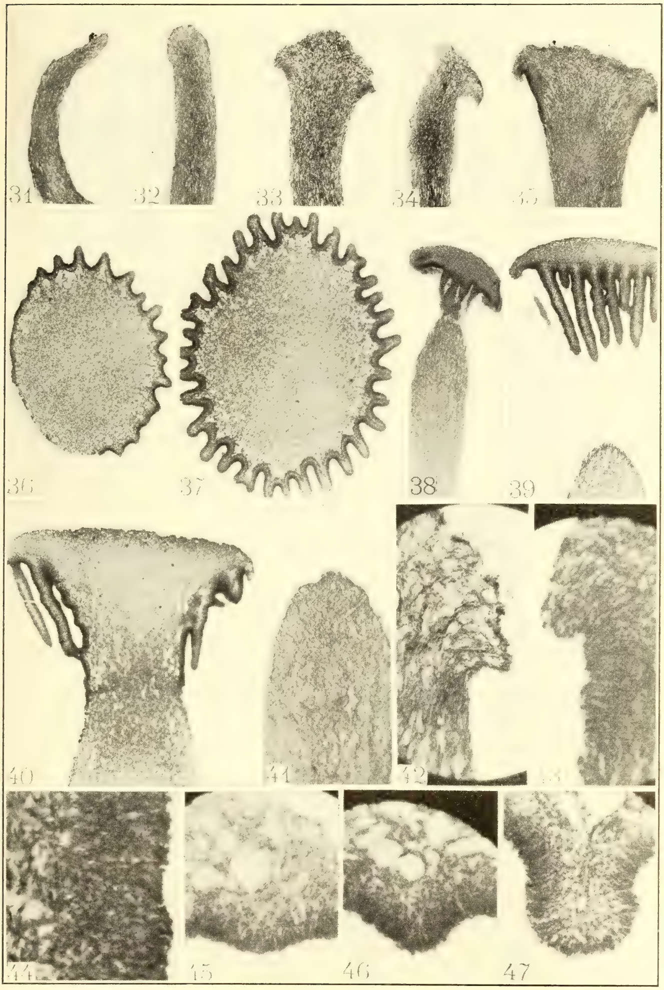
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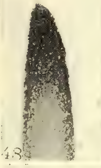
Blizzard: Clitocybe adirondackensis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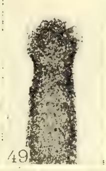
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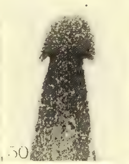
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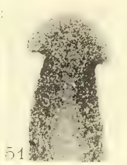
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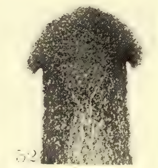
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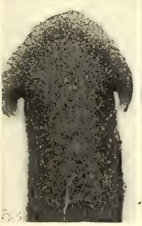
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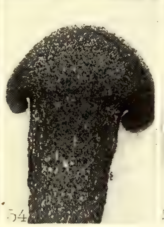
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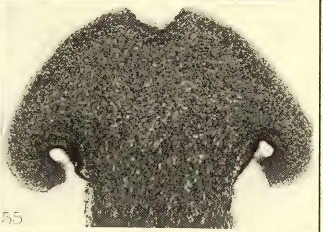
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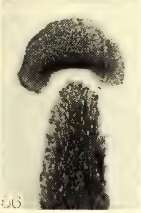
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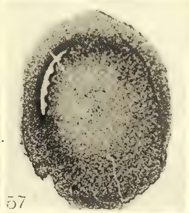
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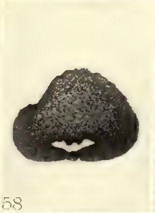
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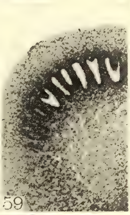
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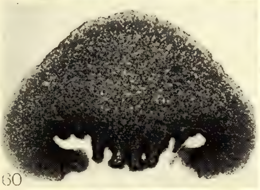
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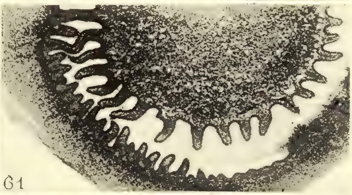
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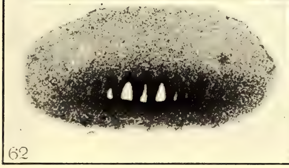
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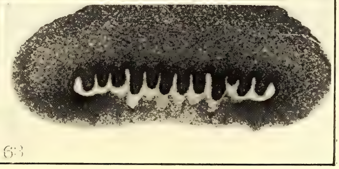
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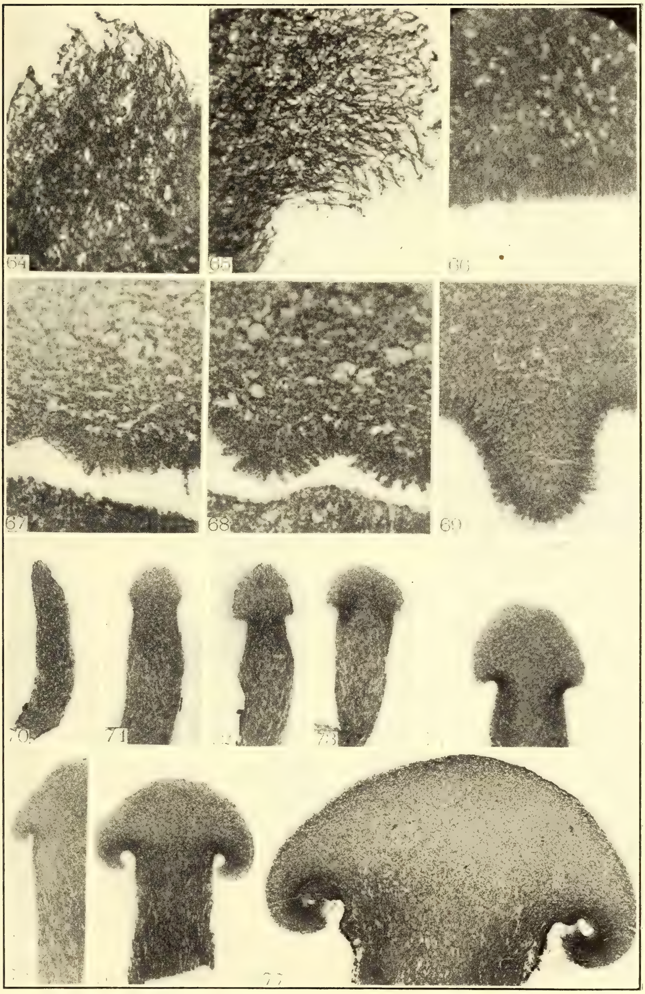
BLIZZARD: CLITOFILUS NOVEBORACENSIS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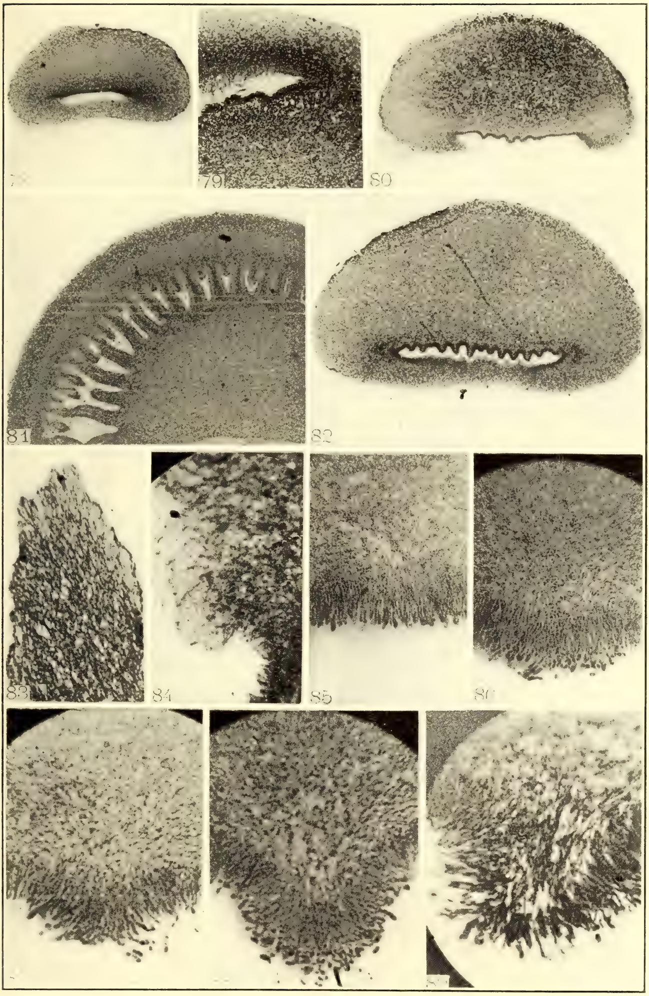
BLIZZARD: CLITOPILUS NOVEBORACENSIS AND CLITOCYBE CERUSSATA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BLIZZARD: CLITOCYBE CERUSSATA

THE ORIGIN AND DEVELOPMENT OF THE GALLS PRODUCED BY TWO CEDAR RUST FUNGI

J. L. Weimer

The question of the origin of the outgrowths caused by *Gymnosporangium Juniperi-virginianae* Schwein. and *Gymnosporangium globosum* Farlow on *Juniperus virginiana* L. has never been settled satisfactorily. The galls produced by *G. Juniperi-virginianae* have been studied by several workers but there still exists considerable difference of opinion as to the method of their origin. The excrescences caused by *G. globosum* have been studied but little.

While making observations on these galls incident to the preparation of another paper the writer became interested in their method of origin. Observations were made throughout two summers and the earliest stages of the development of these galls were studied in the field and later microscopical studies were made. The results of these observations and studies together with a résumé of the literature on the subject are given below.

G. JUNIPERI-VIRGINIANAE

Farlow (1880) states that prior to the time of writing it had been generally accepted that the cedar apples originated in the young cedar stems but that so far as he could ascertain they were deformed leaves. Sanford (1888) studied the pathological histology of the galls produced by this fungus and decided that the galls are modified cedar leaves, while Wörnle (1894) after also studying these galls histologically concluded that they originated from the stem. Heald (1909) thinks that the cedar apples originate from the stem in the axis of a leaf. Kern (1911) places *G. Juniperi-virginianae* among the foliage inhabiting species and Coons (1912) states that while he has never observed or produced infection artificially it is evidently a leaf infection. Reed and Crabill (1915) claim that the cedar apple is nothing but a hypertrophy of a cedar leaf infected by the fungus *G. Juniperi-virginianae.* Giddings and Berg (1915) picture minute galls situated near the end of cedar leaves, hence apparently originating from the leaf. Steward

(1915), after having studied the histology of these growths, concludes that they originate as modified axillary buds; the leaf tissue becoming involved later.

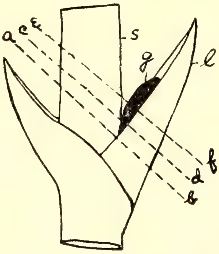
The writer's observations go to show that the cedar apples caused by this species usually break through the upper or inner side of the leaves, the first evidence of infection being the discoloration of the whole or a part of a leaf, followed later by a swelling usually from the upper surface but more rarely from the sides. The young galls grow rapidly and assume the characteristic shape and color very early in their development. It was found that when the infected leaves were removed the galls remained attached (Pl. XII, Fig. 1). This led to the belief that they must be in very close association with the leaf and perhaps originate from it. Specimens such as are pictured by Giddings and Berg (1915) and Coons (1912), where the galls are located near or even beyond the center of the leaf, were found in considerable abundance (Pl. XII, Figs. 2 and 3). This strengthened the theory that these galls originate from the leaf. If these galls originated in the stem or as modified axillary buds with separate fibro-vascular systems it would be reasonable to suspect that in the very young stages at least the gall would be more firmly attached to the stem than to the leaf. A single gall has been found by the writer which has the appearance of having originated from the stem and it may be true that this mode of origin also exists, although it is certainly not the common method about Ithaca, New York. The writer has had the privilege of examining young galls from West Virginia and Wisconsin and the method of origin herein described was also found in those galls.

Before proceeding with a discussion of the internal anatomy of these galls a brief description of the structure of the healthy cedar leaf and stem will be given. The cedar leaf is attached to the stem throughout a large part of its length, only the apical portion being free. In cross section the leaf is triangular in outline at the apex but gradually becomes four-sided toward the base. The epidermis consists of a single layer of somewhat flattened, elongate cells with the outer wall covered by a thick layer of cutin. The epidermal layer on the upper or inner side of the leaf is broken by numerous stomata. Beneath the epidermal layer is a hypodermis on all the sides except the upper. For the most part this consists of a single layer of sclerenchymatous cells. This may however be reinforced at certain places, principally at the corners and in the region of the resin duct, by additional cells of the same character. The central part of the leaf is occupied by a single fibro-vascular bundle of the collateral type. This is composed of a small group of scalariform tracheids and a group of phloem cells about equal in size. Just back of this bundle near the base of the leaf is a resin duct. The remainder of the tissue of the leaf is made up of parenchyma cells. The parenchyma cells near the upper or inner surface below the stomata are globose in shape and are loosely arranged, forming a tissue similar to mesophyll in appearance. The outer layers of cells are elongate, the long axis being perpendicular to the surface forming a palisade tissue. The structure of the very young stems which bear the young cedar apples is only slightly different from that of the leaf except of course that the fibrovascular system consists of a medullated central cylinder which is split up into several collateral bundles by the presence of leaf gaps. The cortical tissue of the stem and the parenchyma cells of the leaf are so much alike that it is impossible to distinguish between them. The parts of the stem not covered by leaves are protected by an epidermal layer similar to that of the leaf.

One of the first and most conspicuous things which may be observed in a longitudinal section of a stem bearing a young gall (Pl. XII, Fig. 4) is the position of the gall as compared with that of the opposite leaf. It is evident in every case that the gall occupies a position identical with that of the leaf on the opposite side of the stem. There is no sign of an axillary structure of any kind. Usually the leaf whose position the gall occupies and on which it develops becomes distorted beyond recognition except that there is evident a portion of its tip. A section through the leaf bundle at the base of the gall shows clearly that the vascular bundles of the gall arise from this leaf bundle. This is best studied in galls which have originated some distance from the axil of the leaf as shown in Plate XIII, Figures 1 and 2. In these figures it will be seen that the gall has been formed by the production of a large number of parenchyma cells from the parenchyma of the leaf, and by the vascular bundles which have arisen from the leaf-trace bundle. Examination of serial sections of such a gall precludes the possibility of the existence of any separate vascular bundle in the leaf from which the gall bundles might have arisen. In cases where the gall lies at or near the base of the leaf and from external appearances might possibly be axial in nature, serial sections show no vascular supply derived from the stele except the normal small leaf trace or its modification. Stewart (1915) decided that the gall bundles are derived from the central cylinder entirely separate from and above the leaf-trace bundle. He illustrates this in Figure 1 of his paper, where he shows at K a section of an axillary bud from which he states the cedar apple is formed. The writer has in only one case found a structure similar to that represented by Stewart. In this case (Pl. XV, Fig. 1b) the structure in question is a section of one side of a terminal bud. An examination of all the sections in the series reveals the presence of the embryonic leaves. The young gall (*g*) beside this bud shows distinctly the difference in the appearance of a true gall and a bud. Evidently Stewart has mistaken a normal axillary bud for a young gall. The writer was permitted to examine some of Stewart's slides and this convinced him that Stewart was mistaken in thinking these structures to be young galls. A careful search of these slides failed to reveal the presence of mycelium in the buds. Stewart admits that "the fungus has not entered the stem at this stage," but concludes that these axillary buds are young galls because structurally these two seem to him alike. So far as seen by the writer this worker's sections show no cases which, when carefully interpreted, as discussed below, demonstrate the axial nature of the gall.

The excrescence caused by *G. Juniperi-virginianae* in its earliest stages consists simply of a few large parenchyma cells similar to those of the leaf. Often no distinct epidermal tissue is apparent at this stage but before the galls enter the winter condition a few layers of cork cells are laid down. The time at which this exterior covering is formed varies in different galls. The beginning of such a layer of cork cells is evident in some very young stages while in other cases galls nearly mature show almost no sign of its development.

That the fibro-vascular system of the gall originates from that of the leaf is evident from the study of the very young stages. How this takes place may be seen in Plate XIII, Figures 1 and 2. The leaf-trace bundle first shows an increase in size beneath the enlarged portion of the leaf. Soon strands of vascular tissue are found leaving the leaf bundle at almost an angle of ninety degrees and passing into the young gall. The vascular tissue of the gall develops rapidly and very early in the development there is present a large amount of conductive tissue in the gall. This same method of origin of the vascular tissue of the gall can be traced in those growths which occur near the base of the leaf. In this case, however, the leaf-trace bundle is very materially affected by the gall and it soon becomes developed to such an abnormal extent that its identity is nearly or quite lost. Reed and Crabill (1915) give a good diagrammatic drawing of the bundle of a young gall originating near the stem. Stewart (1915) thinks that had Reed and Crabill made transverse instead of longitudinal sections of this infected leaf they would have found two bundles entering the gall rather than one. Such sections of numerous galls have been made by the writer and in no case has more than the one abnormally large bundle been found. In Plate XIV, Figures 1, 2, and 3 are shown sections from a series cut from an infected leaf, the gall being formed near the axil. A section taken a little way above the junction of the leaf and stem is represented in Figure 1 (line *a–b*, text figure 1). Here it is evident that the vascular bundle has been affected since it has more than doubled in size. A section taken farther from the stem is illustrated in Figure 2 (line *c–d*, text figure 1). This shows the bundle split into three parts by the intercalary formation of large cells filled with resin. These segments of the vascular system later branch out and become diffused throughout the gall (Fig. 3) (line *e–f*, text figure 1). A photograph of a stage somewhat comparable to Stewart's text figure is shown in Plate XIV, Figure 4. The central cylinder of the stem is shown at *a* and passing off from this is the greatly enlarged and modified leaf trace bundle breaking up and passing out in all directions in the gall. In Plate XIII, Figure 3, is shown a transverse section of the stem at *s* with two leaves at *l* and *l′*. From one side of leaf *l* a gall (*g*) is being produced. The vascular system of leaf *l* is much enlarged and from it strands of vascular tissue (*v*) extend into the gall (*g*). These figures check the opinion of Reed and Craybill concerning the single bundle supply. In cases where the gall occurs near the leaf base the increase in vascular tissue occasioned by its presence enlarges the leaf trace even through the cortex. At the base of the gall its vascular tissue frequently takes the form of an irregular hollow cylinder



TEXT-FIG. 1. Diagrammatic drawing of a portion of a cedar twig with two leaves attached. Lines *a–b*, *c–d*, and *e–f* show the approximate positions from which the sections illustrated in Plate XIV, Figs. 1, 2 and 3 respectively, were taken. *s*—stem, *g*—gall and *l*—leaf.

simulating that of a branch. To interpret correctly, especially in longitudinal sections, the enlarged and irregular base of the leaf trace (a mass of tissue sometimes even near its base partially broken up, and dividing soon into two masses, the larger upper one simulating a branch stele) serial sections are clearly absolutely essential. It is quite probable that Stewart has drawn his conclusions from individual sections. It is easy to see, further, how in this case a longitudinal section that is not quite median might lead to erroneous conclusions.

Sanford describes exactly the same condition that the writer has found in numerous cases. The writer therefore concludes with the majority of investigators along this line that in most cases at least and probably in all cases the gall is foliar, and does not represent a transformed branch.

G. GLOBOSUM

There has been no controversy in regard to the origin of the gall produced by *G. globosum*. Heretofore most workers have assumed from the external appearance of the old galls that they originate in the stem. Farlow (1880) who first named this fungus states that unlike *G. Juniperi-virginianae* it does not break through the central part of the leaf, but bursts through the stem at the point of attachment of the leaves. Pammel (1905) states that the galls break through the stem where the leaf is attached. Kern (1911) described the telial stage of this species, as being caulicolous. Stewart (1915) gives an account of histological studies made which he interpreted as showing beyond a doubt that this cedar gall originates from the limb as has always been supposed.

In order to make more careful observations on this subject a small cedar tree about four feet high and bearing numerous cedar apples was selected and all the galls removed early in April (1914) in order that they might not be confused with other galls appearing later.

This tree was kept under close observation and on July 25 the first young gall was visible. No aecia were mature at this time. The young galls seemed to be composed of modified portions of leaf rather than stem tissue. These galls were tagged and their development followed throughout the summers of 1914 and 1915. They grew very slowly and in late autumn were not more than two millimeters in diameter. The following spring (1915) these cedar apples sporulated, thus showing that this fungus, like *G. Juniperi-virginianae*, requires nearly two years for the completion of its life cycle.

On March 19, 1915, several small cedar trees were planted in pots in the greenhouse and on April 7 several leaves were found on these trees from the surface of which telial horns were developing (Pl. XV, Fig. 2). One or more were seen to come from the upper surface of the infected leaves which were swollen very little or not at all. These telial horns resembled those of *G. globosum* in shape and color and the spore measurements corresponded to those of that species. Inoculations were made on Crataegus leaves with some of these spores and the characteristic aecia of *G. globosum* developed; thus showing that the original determination was correct. Later similar specimens were found in nature. Often the infected leaves are yellowed throughout a certain portion of their length and the telial horns develop from those discolored areas. These tentacles may be found developing from any part of the upper surface or side of the leaf. Sections of some of these leaves showed them to be completely permeated with mycelium which in some cases at least did not extend to the base of the leaf. Infection must have undoubtedly occurred in the leaf.

Having observed that galls of *G. globosum* sometimes originate in the leaf, more careful observations were made to determine if possible whether this is always true. A great number of galls of this species were examined both during the autumn and winter of 1914 and 1915 and during the summer of 1915. Hundreds of galls were examined and in every case the foliar origin was found. These galls, however, usually develop near the base of the leaf and displace a certain part of it. As the galls continue to develop the terminal portion of the leaf remains attached to the gall and may be found here for some time. A careful study of Plate XV, Figs. 3 and 4 will make this point clear. A large amount of variation occurs. In some cases the gall may grow out from the upper surface of the leaf as do the galls caused by *G. Juniperi-virginianae*, or they may burst out of the side. A close inspection of older galls showed in nearly every case the dead tip of the original leaf still intact (Pl. XV, Figs. 3, 4, 5 and 6).

The gall grows slowly and is perennial, forming spores for several years. In the early stages these galls are nearly mahogany red in color as compared with the green color of the minute galls of *G. Juniperi-virginianae*. The red color gradually changes to grayish brown in the older galls. The shape of these galls is more or less globose from the beginning and often flattened on the side next to the stem (Pl. XV, Fig. 4). When the gall becomes older, it displaces the leaf as stated above and as it continues to develop from year to year it becomes firmly attached to the twig, appearing to have originated in the twig (Pl. XV, Fig. 5 and Pl. XVI, Fig. 3).

In case of the above mentioned infected leaves where there was scarcely any swelling, the infection presumably took place in the summer of 1913 but was not apparent in the late summer or fall of 1914 and first became obvious in the spring of 1915. That the fungus had been developing in the leaf for some time seems certain when it is considered that in nineteen days after the trees were removed to the greenhouse telial horns had been produced. For some unknown reason the characteristic stimulation of cellular activity did not occur and when the mycelium reached the spore-bearing age, spores were produced.

Other cedar trees brought into the greenhouse early in the spring of 1914 produced cedar apples during the spring of 1915. These were scarcely more than telial horns coming directly from the leaf as in the other cases described. These were probably infected in the fall of 1913 and the mycelium was able to live in the leaf from that time until the spring of 1915, or approximately two years before causing any noticeable effect on the host.

These small galls developing on the leaf at considerable distance from the stem seldom reach any great size, probably due to their distance from the stem and a consequent lack of sufficient vascular tissue development.

A study of a large number of serial sections through the stem and young gall shows a condition such as is apparent in Plate XVI, Figures 1, 2, and 3. Plate XV, Figure 7, shows a section of a cedar leaf which had a slight discoloration but almost no swelling. The leaf when sectioned was found to be permeated with mycelium. A corky exterior layer K is already being developed in the gall shown in Plate XVI, Figure 1. The resin duct *r* is present and the vascular bundle is the leaf-trace bundle somewhat enlarged. Figure 2 shows much the same condition. Figure 3 illustrates a still more advanced stage. In this section the tip of the old leaf still remains visible at the apex and the corky exterior covering is well developed. The gall has become closely attached to the stem similar to the condition found in old galls where the stem tissue is probably also involved.

SUMMARY

The galls produced by *G. Juniperi-virginianae* and *G. globosum* on *Juniperus virginiana* originate as modified leaves.

The vascular systems of the galls are composed of the enlarged and modified leaf-trace bundles.

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DEPARTMENT OF PLANT PATHOLOGY,
CORNELL UNIVERSITY

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EXPLANATION OF PLATES XII–XVI

PLATE XII

FIG. 1. Young galls caused by *Gymnosporangium Juniperi-virginianae* showing their axillary position and their relation to the leaf. The two galls at the right were removed by pulling on the tips of the leaves to which they are attached. Compare the method of origin here with that shown for *G. globosum* galls in Pl. XV, Fig. 3.

FIG. 2. Mature gall of *G. Juniperi-virginianae* developed from the upper surface of the leaf and producing one telial horn.

FIG. 3. Three mature galls of *G. Juniperi virginianae* with telial horns partly gelatinized. These galls have evidently developed from the upper side of the leaves upon which they occur.

FIG. 4. Longitudinal section of stem and leaves of young cedar twig showing the relation of the gall (*g*) to the leaf (*l*) which bears it and to the leaf on the opposite side of the stem.

PLATE XIII

FIG. 1. Young gall (*g*) forming on the leaf (*l*) at a considerable distance from the stem (*s*). The vascular tissue in the young gall is very abundant and arises from the leaf-trace bundle (*t*).

FIG. 2. A young gall borne near the tip of the leaf showing the vascular development as in Fig. 1. The letters correspond to those in Fig. 1. The connection of the vascular tissue is more readily visible.

FIG. 3. A transverse section of a stem (*s*) with two opposite leaves (*l* and *l'*). A gall (*g*) has developed from the side of leaf *l* and vascular strands (*v*) are derived from the enlarged leaf-trace bundle at *t*.

PLATE XIV

FIG. 1. Section through a leaf (*l*) with a basal gall, the section taken as shown in diagram and transverse to the leaf trace. The vascular bundle (*v*) is considerably enlarged. Only one bundle is present, supplying both leaf and gall. This precludes the possibility of a separate origin of the vascular system of the gall, *i. e.*, of the axial nature of the latter. (See text figure 1.)

FIG. 2. Section of the same leaf (*l*) as shown in Fig. 1 but taken farther from the stem (*s*). The vascular bundle has broken into three distinct segments.

FIG. 3. Section from the same leaf as in Figs. 1 and 2 but taken still farther from the stem. Here the vascular tissue has become much diffused.

FIG. 4. Transverse section of a medium-sized gall (*g*) and the stem which bears it (*s*). The leaf on the opposite side of the stem is shown at *l*. The vascular tissue of the gall originates as one large strand at *a* which finally breaks up into a fan-like system of bundles. How this takes place is made clear by a careful study of Figs. 1, 2, and 3.

PLATE XV

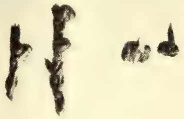
FIG. 1. Longitudinal section of young stem (*s*) showing terminal bud (*b*), young gall (*g*) and leaves (*l*). The bud (*b*) has identically the same appearance as the young gall shown by Stewart (1915) in Fig. 1 of his paper. There is no mycelium in this bud while mycelium is abundant in the gall beside it.

FIG. 2. Telial horns of *G. globosum* issuing directly from the leaf.

FIGS. 3 AND 4. Young galls of *G. globosum* originating from leaves, the tips of which are apparent at the top of the galls. The white appearance of the upper portion of the galls is due to fragments of the leaf tissue.

FIGS. 5 AND 6. Mature galls showing the remains of the leaves from which they originated. The galls shown in Fig. 5 have fruited more than once.

FIG. 7. A transverse section of a leaf which was slightly discolored and very slightly swollen at the base. The leaf is permeated with mycelium throughout nearly



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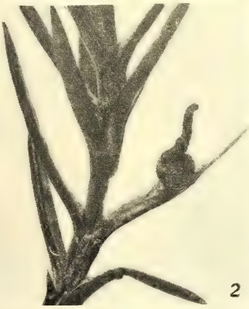

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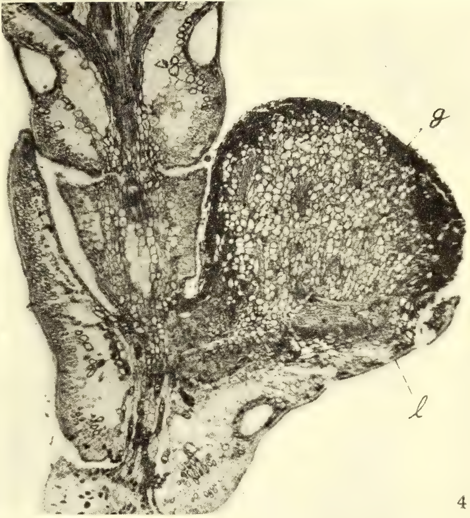

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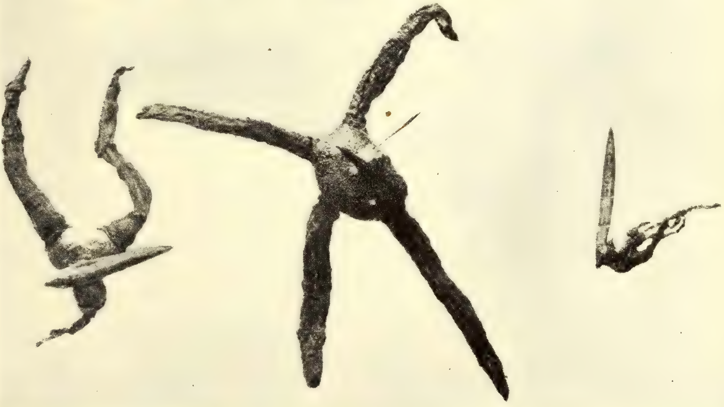

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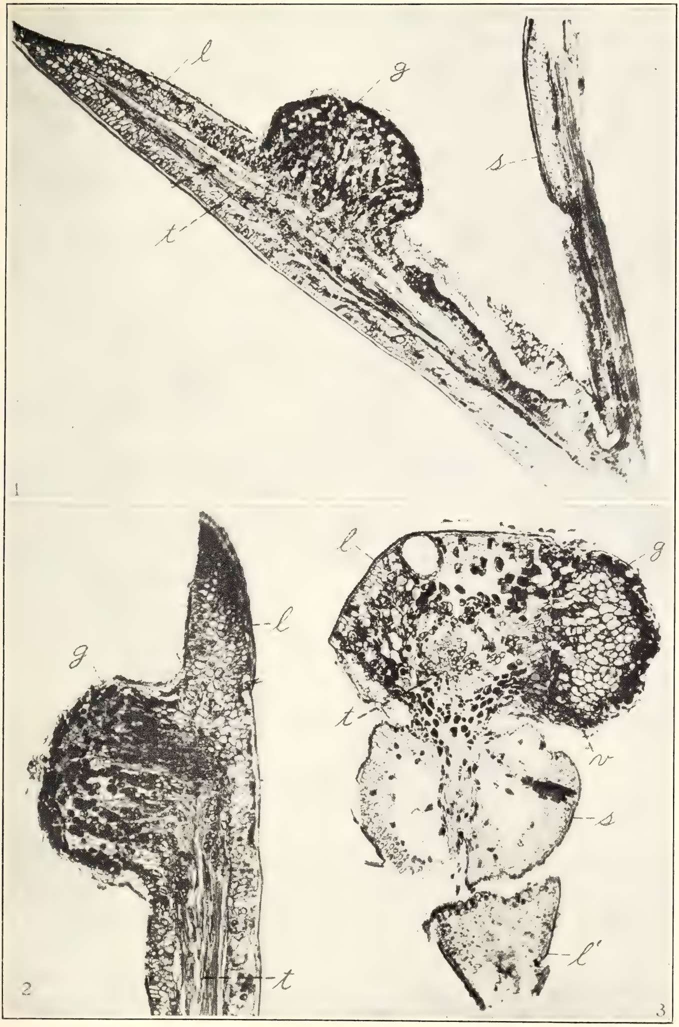
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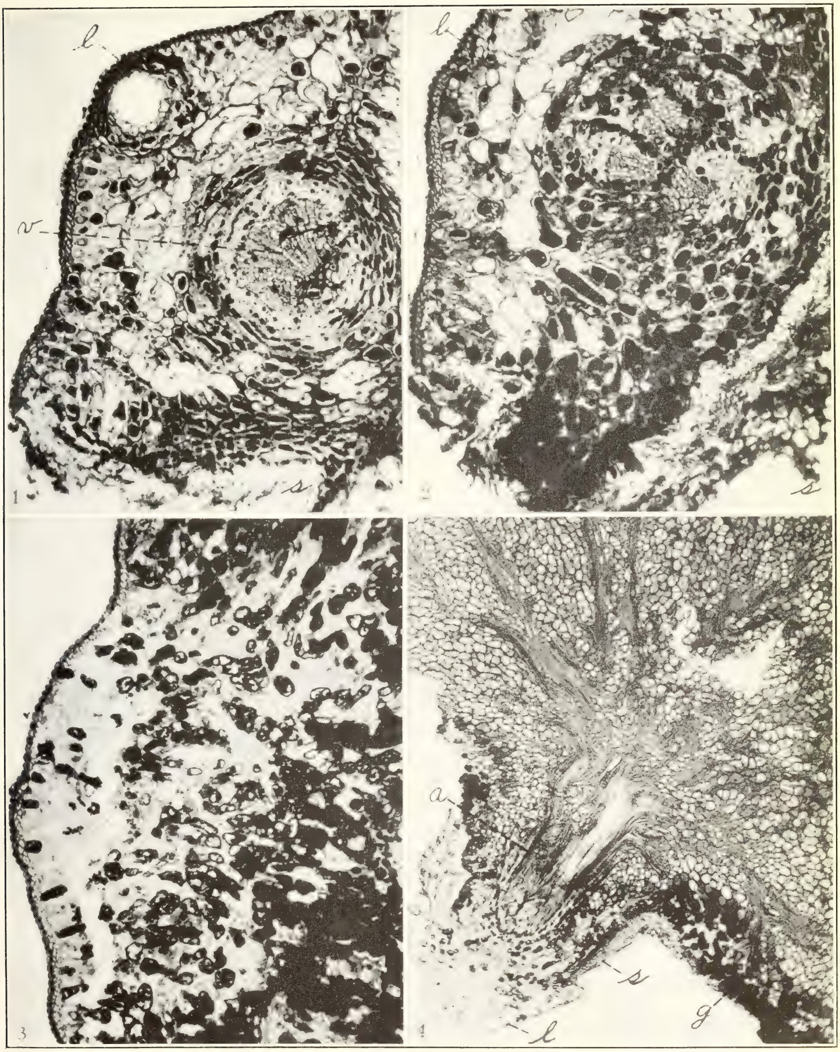
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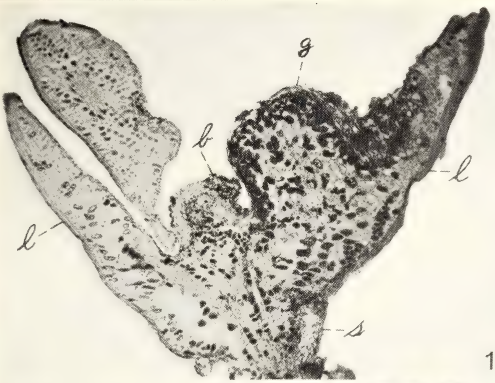

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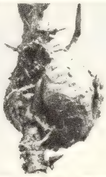

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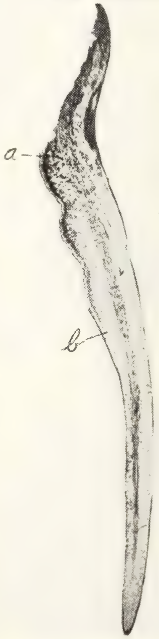

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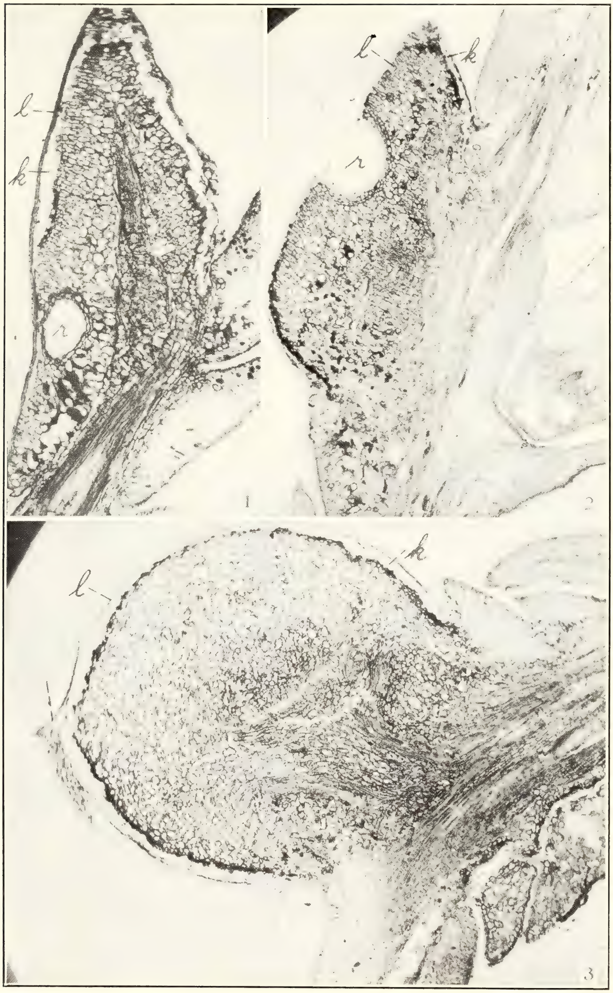
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WEIMER: GALLS PRODUCED BY CEDAR RUST FUNGI

its entire length. A layer of cork has been laid down in the cortical tissue as denoted by the dark line extending from the base to about the center of the leaf (see *a–b* in photograph).

PLATE XVI

FIGS. 1 AND 2. Sections of leaves (*l*) affected with *G. globosum* showing resin ducts (*r*) and their relation to the stems. The white area beneath the epidermis (*k*) in both galls is the corky covering which develops very early in galls caused by this fungus.

FIG. 3. Section of a gall in a more advanced stage than represented in Figs. 1 and 2. The tip of the leaf is evident at *l* and the corky layer (*K*) surrounds the gall on all free sides. The gall is firmly attached to the stem and it can easily be seen how the condition shown in Pl. XV, Fig. 5, develops.

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CONTENTS

The perennial scapose Drabas of North America. . EDWIN BLAKE PAYSON 253

The osmotic concentration of the tissue fluids of Jamaican montane rain-forest vegetation. . . . . . . J. ARTHUR HARRIS AND JOHN V. LAWRENCE 268

The viability of radish seeds (Raphinus sativus L.) as affected by high temperatures and water content . . . . H. D. WAGGONER 299

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THE PERENNIAL SCAPOSE DRABAS OF NORTH AMERICA

Edwin Blake Payson

The treatment of the group of Drabas here presented grew out of an attempt to name some seven members of this group collected in the summer of 1916 in the mountains of central Idaho by J. F. Macbride and the author. It soon became evident that available literature was entirely inadequate and that either specific lines were extremely hard to define or that a number of species were being treated under comparatively few names. Careful study of adequate herbarium material failed to show intermediate forms and it was noticed that each form (of which several specimens could usually be found) was restricted to a rather limited range. Differences that at first sight seemed inconsequential proved to be constant. The conclusion was obvious that there were within this group many distinct and easily separable species. That they should have long passed for a few was not strange when their diminutive size was considered. Practically all are plants of arctic or alpine habitats and this alone might account for their very similar aspect. Under similar conditions the various species have developed along analogous lines. The author believes that with the recognition of many instead of few species practically all difficulty in the determination of these plants will disappear.

In the twenty-six species comprising this group three are circumpolar and are also found in the main chain of the Rocky Mountains. One is a local species from the region of Hudson Bay. Twenty-two are peculiar to western North America and their distribution will be considered in detail. Seven have been found in the Rocky Mountains proper and, of these seven, two have possibly been developed farther westward and have migrated east. Fifteen are truly western, since no representatives have been reported from the Rocky Mountains. Of these fifteen, four are peculiar to Idaho and seven are found farther west in Nevada, California and Oregon. Sixteen of the twenty-two are not found as far north as the southern boundary of Washington and perhaps none extend farther south than the southern boundary of Colorado and Utah. It can thus be seen that the great multiplication of species occurs between the thirty-seventh and forty-sixth parallels of north latitude and west of the main chain of the Rocky Mountains. This region is characterized by many more or less isolated mountain ranges, ranges often separated by many miles of low territory. Since climatic and edaphic conditions are in general very similar in most alpine regions and the specific distinctions are not of such a nature as to be considered adaptive, we can scarcely believe that the differentiation of species has come about by selection. There is, however, a close relation between isolation and the multiplication of species and this factor must be considered seriously in any attempt to account for the evolution of this group.

Characters that do not vary with ecological conditions and remain constant in a given species are often quite different in different families and even genera. Of paramount importance to the systematist in these Drabas is the character of the pubescence. Classification must always be based primarily upon this character. The uniformity of the pubescence within a species is most interesting and the use of a lens or even a compound microscope is imperative. Some species never bear truly stellate hairs, as for example, *D. alpina* and *D. fladnizensis*. One very interesting species bears no other hairs on the leaves except strong marginal cilia. In some species no cilia are present and the leaf surface is covered with sessile stellate hairs as in *Lesquerella*. *D. cruciata* and *D. asterophora* are of especial interest because of the prevalence of cruciform hairs. Nearly every species might be determined by the character of the pubescence alone. Other characters of considerable importance are the presence or absence of pubescence on the pods, the length of the style, the shape of the pod, the shape of the leaf and the form of the fruiting inflorescence.

The material cited under the specific names may be found in the Rocky Mountain Herbarium at the University of Wyoming and in the herbarium of the Missouri Botanical Garden at St. Louis. To the curators of these institutions the author wishes to express his gratitude.

Much of the material seen has been examined by Dr. E. Gilg. He used a number of herbarium names which have unfortunately never been published and there is some confusion as to his conception of types. His determinations were very valuable and often served to corroborate the author's opinions. The types of the species published by Macbride and Payson are to be found in the Gray Herbarium at Harvard; those published by the author alone are in the Rocky Mountain Herbarium, with the exception of *D. Mulfordae,* which is at St. Louis. Some names are omitted from this treatment that might be expected here. *D. crassifolia* Graham is scapose but belongs rather to the biennial class than to these densely cespitose plants. The identity of *D. densifolia* Nutt. remains obscure. Material labeled as typical in the Gray Herbarium seems referable to *D. oligosperma. Draba eurycarpa* Gray has long been considered related to this group of plants, but there seems to be some doubt about it being a Draba.

Key to the American Perennial Scapose Drabas

Leaves bearing no stellate or conspicuously branching hairs.
Leaves densely imbricated, forming compact, subspherical tufts on the ends of the caudex branches, pods corymbose.
Pods glabrous . . . . 4. *D. globosa.*
Pods pubescent, more densely cespitose . . . . 5. *D. sphaerula.*
Leaves not so densely imbricated, scapes mostly over 1 cm. long.
Style very short, stigma sessile or subsessile.
Pods racemose, flowers yellow . . . . 1. *D. alpina.*
Pods corymbose, flowers white . . . . 2. *D. fladnizensis.*
Style evident, .5 mm. or more long.
Pods glabrous.
Leaves 5–6 mm. long, obovate . . . . 15. *D. cylomorpha.*
Leaves 2–3 mm. long, pods narrowly elliptical . . . . . 3. *D. oreibata.*
Pods pubescent.
Leaves broadly oblanceolate . . . . 16. *D. Lemmoni.*
Leaves linear, midvein prominent.
Leaves glabrous except for strong cilia on the margins . . . . 7. *D. Nelsonii.*
Leaves pubescent, cilia weak . . . . 6. *D. Douglasii.*
Leaves bearing some stellate or branching hairs.
Pods glabrous.
Style less than .5 mm. long.
Pods linear (1 cm. or more long) . . . . 13. *D. lonchocarpa.*
Pods broader, less than 1 cm. long.
Leaves stellate . . . . 12. *D. nivalis.*

Leaves with simple or branching hairs............1. *D. alpina.*
Style evident (over .5 mm. long).
Pods globose..................................22. *D. sphaeroides.*
Pods flattened.
Pods 3–4 mm. long.
Leaves obovate..........................21. *D. uncinalis.*
Leaves linear.............................9. *D. oligosperma.*
Pods over 5 mm. long.
Leaves broadly linear....................14. *D. laevicapsula.*
Leaves broadly spatulate or oblanceolate.
Leaves densely stellate and silvery.......24. *D. argyrea.*
Leaves more sparsely pubescent, green.
Pods narrowly lanceolate, not over 2 mm. wide.......................20. *D. cruciata.*
Pods broader (3 mm. or more).
Hairs mostly cruciform...........17. *D. asterophora.*
Hairs simple or branching..........1. *D. alpina.*
Pods pubescent.
Seeds winged, leaves silvery........................23. *D. pterosperma.*
Seeds not winged.
Pods globose, stellate pubescent..................25. *D. sphaerocarpa.*
Pods more or less flattened.
Leaves densely imbricated, not over 5 mm. long, silvery stellate, cilia absent..................26. *D. subsessilis.*
Leaves not so densely imbricated, mostly over 5 mm. long.
Leaves linear or slightly spatulate.
Fruiting raceme corymbose.............11. *D. vestita.*
Fruiting raceme elongated.
Stellate hairs sessile, near apex of leaf...8. *D. oligosperma.*
Stellate hairs not sessile nor usually restricted to apex of leaf.
Ciliations not conspicuous, plants not soboliferous.................9. *D. incerta.*
Ciliations conspicuous, plants soboliferous.....................18. *D. Mulfordae.*
Leaves not linear.
Leaves silvery-white, entirely stellate.....24. *D. argyrea.*
Leaves greenish, or if silvery, cilia evident.
Pods narrowly lanceolate............20. *D. cruciata.*
Pods broader (3 mm. or more).
Style .5 mm. long, plants of Hudson Bay.................10. *D. Bellii.*
Style 1 mm. long or more (usually).
Ciliations numerous and conspicuous..................18. *D. Mulfordae.*

Ciliations absent or inconspicuous . . . . 19. *D. ventosa.*

1. D. ALPINA L. Sp. Pl. 642. 1753.

A circumpolar species probably never found within the United States.

Specimens Examined.—ALASKA: Hall Island, July 14, 1899, *Trelease & Saunders*, no. 3922; same locality, July 14, 1899, *Trelease & Saunders*, no. 3924.

2. D. FLADNIZENSIS Wulf. in Jacq. Misc. Austr. 1: 147. 1778.

Specimens Examined.—CANADA: Digges Island, Hudson Strait, Sept. 15, 1884, *R. Bell;* Okkak, N. E. Labrador, *G. Auspach;* Table Top Mt., Gaspe Co., Quebec, Aug. 10, 1881, *J. A. Allen;* Nottingham Island, Hudson Bay, Aug. 26, 1884, *R. Bell.* COLORADO: Gray's Peak and vicinity, Aug. 6, 1885, *H. N. Patterson*, no. 3; Sawatch Range, *Brandegee*, no. 13, 713. GREENLAND: Prakes Fiord, *W. H. Burk*, no. 10, 1891; Disco, Ivannersoil, June 22, 1871, *Th. M. Fries.* UTAH: Mt. Barette, July 26, 1905, *Rydberg & Carlton*, no. 7240.

3. **Draba oreibata** Macbride & Payson, n. sp.

Cespitose perennial: leaves oblong-linear, obtuse, 4–6 mm. long, midvein evident, glabrous except for unbranched cilia on margins: scapes leafless, slender, glabrous, 3–5 cm. long: sepals glabrous, yellowish; petals white, twice as long as the sepals: fruiting inflorescence elongated: pods flat, glabrous, narrowly elliptical, 8–10 mm. long, 2–3 mm. broad; style slender, nearly 1 mm. long.

D. oreibata probably finds its closest relative in *D. fladnizensis.* From this it may be separated by the shorter, more obtuse leaves, the elongated instead of the corymbose fruiting inflorescence, the elliptical instead of lanceolate pod and especially by the slender style. In *D. fladnizensis* the stigma is sessile or nearly so.

Specimens Examined.—IDAHO: alpine summit of Lost River Mts., west of Clyde, Blaine Co., July 10, 1916, *Macbride & Payson*, no. 3135 (type in Gray Herbarium, duplicate in Ry. Mt. Herbarium). UTAH: southern Utah, May 14, 1874, *A. L. Siler;* southern Utah (near Osmer), *A. H. Siler*, no. 34, southern Utah, 1874, *Parry*, no. 34.

4. **Draba globosa** Payson, n. sp.

Densely cespitose perennial; caudex much branched: leaves densely imbricated, forming globose tufts on the ends of the caudex-branches, scarcely over 5 mm. long, broadly linear, acute, midvein evident, glabrous except for short, unbranched marginal cilia: scapes glabrous, rather stout, scarcely 1 cm. long: flowers unknown: fruiting inflorescence corymbose: pods 4–6, broadly lanceolate, flattened, glabrous, about 5 mm. long, 3 mm. wide; style stout, scarcely 1 mm. long: seeds 2–4 in each cell, not winged.

This plant was evidently recognized as distinct by Dr. E. Gilg for I find some specimens in the Rocky Mt. Herbarium labeled by him with a name which has never been published. The aspect of this species and the next suggest *D. subsessilis* Watson. This resemblance is however probably not indicative of any real relationship.

Specimens Examined.—UTAH: Fish Lake, Uintah Mts., June 17, 1902, *Goodding;* Little Cottonwood Canyon, Salt Lake Co., Aug. 3, 1904, *Garrett*, no. 1555; Alta, Wasatch Mts., Aug. 12, 1879, *Jones*, no. 1235. WYOMING: La Plata Mines, Snowy Range, Aug. 29, 1898, *E. Nelson*, no. 5246*a* (type in Ry. Mt. Herbarium). The type was found on the same sheet with two specimens of "*D. andina*" and was given an "a" number. Other sheets of this collection in various herbaria may be found also to bear specimens of *D. globosa.*

5. **Draba sphaerula** Macbride & Payson, n. sp.

Densely pulvinate-cespitose perennial; caudex much branched: leaves about 2 mm. long, densely clustered on the ends of the caudex branches into small, compactly imbricated spheres, glabrous except for the unbranched marginal ciliae, broadly linear, acute, midvein obscure: scapes barely rising above the leaves, pubescent: sepals pubescent, yellowish; petals yellow, exceeding the sepals but little: fruiting inflorescence corymbose: pods few (1 or 2), broadly lanceolate, about 3 mm. long, flattened, pubescent with stellate or branched pubescence; style evident, less than 1 mm. long: seeds neither winged nor margined.

D. sphaerula is quite closely related to *D. globosa* and further collections may even show the two to merge. It differs from *globosa* in the pubescent instead of the glabrous pods, the smaller, more densely imbricated leaves and the shorter scape. That the very short scapes are not due to ecological factors was evident when the type of *sphaerula* was collected, for it grew among the tufts of *D. Nelsonii* in which the scapes were much elongated.

Collected on an alpine slope near Parker Mt., Custer Co., Idaho, July 17, 1916, *Macbride & Payson*, no. 3336 (type in Gray Herbarium, duplicate in Ry. Mt. Herbarium).

6. D. Douglasii Gray, Proc. Amer. Acad. **7**: 328. 1867.

Braya oregonensis Gray, Proc. Amer. Acad. **17**: 199. 1882.

Cusickia Gray, l. c.

D. Crockeri Lemmon, Bull. Torrey Club **16**: 221. 1889.

Specimens Examined.—California: Sierras of California, *Lemmon.* Oregon: Dry, stony hills, eastern Oregon, May 24, 1898, *Cusick*, no. 1883. Utah: 1858–59, *H. Engelmann.* This speciman is referred here very doubtfully. It is however too immature to make determination certain.

7. **Draba Nelsonii** Macbride & Payson, n. sp.

Cespitose alpine perennial, caudex-branches clothed with dead leaves below; leaves linear, 5–7 mm. long, 1 mm. or less wide, acute, midvein prominent, glabrous except for the strong marginal cilia: scapes slender, glabrous or sparingly pubescent, 1–4 cm. long: sepals greenish, usually glabrous; petals yellow, nearly twice as long as the sepals: fruiting inflorescence elongated: pods 6–15, broadly lanceolate, 3–6 mm. long, 2–3 mm. broad, simply pubescent, flattened: style 1 mm. more or less long: seeds 1–4 in each cell, wingless.

Of all the newly characterized species, *D. Nelsonii* seems to be the most distinct and most widely distributed. Its affinities are undoubtedly with the *oligosperma* group. In manner of growth, elongated raceme and rather small pods it is quite similar to *D. oligosperma* and might even be mistaken for that species except for the total absence of stellate pubescence in *D. Nelsonii.* It is a great pleasure for the authors to dedicate this plant to their friend and teacher, Dr. Aven Nelson, through whose efforts and assistance the expedition was made possible that led to the discovery of this fine species.

Specimens Examined.—California: Castle Peak, Nevada Co., Aug. 3, 1903, *Heller;* Modoc Co., 1898, *Mrs. Bruce;* rocky, exposed ridges, Mt. Stanford, Nevada Co., July 17, 1892, *Sonne*, no. 14. Idaho: exposed alpine summit, Antelope Mts., near Martin, Blaine Co., July 6, 1916, *Macbride & Payson*, no. 3077 (type in Gray Herbarium, duplicate in Ry. Mt. Herbarium); Soldier Mts., near Corral, Blaine Co., June 26, 1916, *Macbride & Payson*, no. 2894; exposed summit near Parker Mt., Custer Co., July 17, 1916, *Macbride & Payson*, no. 3253. Oregon: Blue Mts., July, 1886, *Cusick*, no. 1345. Washington: Yakima Region, 1882, *Brandegee*, no. 373 (in part).

8. D. oligosperma Hook. Fl. Bor. Amer. **1**: 51. 1833.

D. andina (Nutt.) A. Nels. Bull. Torrey Club **26**: 352. 1899.

D. saximontana A. Nels. Bull. Torrey Club **27**: 264. 1900.

D. oligosperma was described as having white flowers and it is certain that in *D. andina* the flowers are yellow. Since there seems to be no other difference, however, the two species are merged.

Specimens Examined.—BRITISH COLUMBIA: Carbonate Draw, July 13, 1904, *J. Macmillan*, no. 297; Canmore, Ry. Mts., June 29, 1885, *Macoun;* Silver City, Ry. Mts., Aug. 3, 1885, *Macoun;* N. Fork of Old Man's River, Aug. 10, 1883, *Macoun.* IDAHO: Sawtooth National Forest, 1910, *C. N. Woods*, no. 80; Boise, *Wilcox.* MONTANA: Monida, Madison Co., June 16, 1899, *A. Nelson & E. Nelson*, no. 5423; Bridger Mts., June 15, 1897, *Rydberg & Bessey*, no. 4181; Bridger Mts., June 4, 1901, *W. W. Jones;* Bridger Canyon, May 15, 1901, *E. J. Moore;* Little Belt Mts., Aug. 10, 1896, *Flodman*, no. 498; Bridger Canyon, Bozeman, May 27, 1899, *Blankinship;* June, 1894, *Mrs. Moore;* Mt. Bridger, June 26, 1899, *Blankinship;* Bridger Mts., June 15, 1897, *Rydberg & Bessey*, no. 4180 (this seems to be nearly typical *oligosperma;* its flowers are white). NEVADA: Ruby Hill, July 7, 1891, *Jones;* Bunker Hill, Toiyabe Range, July 29, 1913, *Kennedy*, no. 4184. UTAH: Logan Peak, Cache Co., July 4, 1910, *Charles Piper Smith*, no. 2245. WYOMING: Bush Ranch, Sweetwater Co., June 10, 1900, *A. Nelson;* Golden Gate, Yellowstone Park, June 28, 1899, *A. Nelson & E. Nelson;* Laramie Hills, Albany Co., June 3, 1900, *A. Nelson*, no. 7019; Laramie Hills, Albany Co., May 30, 1898, *A. Nelson*, no. 4323; Laramie Hills, June 21, 1892, *B. C. Buffum*, no. 65; Kemmerer, June 1, 1907, *A. Nelson*, no. 9027; Laramie Hills, June, 1893, *A. Nelson*, no. 3237; T. B. Ranch, Carbon Co., June 20, 1901, *Goodding*, no. 58; Telephone Mines, Albany Co., Aug. 1, 1900, *A. Nelson*, no. 7873; Laramie Hills, May, 1895, *A. Nelson*, no. 1223; sandy hilltops, Laramie, May 25, 1910, *A. Nelson*, no. 9334; Freezeout Hills, July 10, 1898, *E. Nelson*, no. 4487; La Plata Mines, Aug. 29, 1898, *E. Nelson*, no. 5246; Gros Ventre Fork, June 8, 1860, *Hayden;* West Slope of Wind River Mts., June 6, 1860, *Hayden;* gravelly hills in Wind River Valley, May 15, 1860, *Hayden*; near Mammoth Hot Springs, Yellowstone Park, June, 1893, *Burglehaus;* Yellowstone Park, 1885, *Tweedy*, no. 567; near South Gap, June, 1873, *Parry*, no. 11.

D. pectinata (S. Wats.) Rydb. Bull. Torrey Club **39**: 327. 1912. *D. glacialis* var. *pectinata* S. Wats. Proc. Amer. Acad. **23**: 260. 1888. These seem according to specimens labeled by Rydberg in the Gray Herbarium to be referable to *D. oligosperma.*

9. **Draba incerta** Payson n. sp.

Somewhat cespitose perennial, caudex branching: leaves linear or linear-spatulate, 7–10 mm. long, about 2 mm. wide, not rigid, midvein obscure, greyish with long stellate pubescence and weak marginal cilia: sepals villous; petals yellow, twice as long as the sepals: fruiting inflorescence elongated: pods numerous (6–14), flattened, simply pubescent, broadly lanceolate, 4–6 mm. long: style evident, less than 1 mm. long: seeds neither winged nor margined.

Draba incerta ia a rather unsatisfactory species appearing almost as if it were produced by peculiar ecological conditions. It differs so strikingly in leaf characters from its nearest relative (*D. oligosperma*), however, that one could scarcely consider them identical. I find that Dr. Gilg has evidently considered this as a distinct species, although he has included more than one species under the manuscript name which he gave it. It may be distinguished from *D. oligosperma* by the thinner leaves which are not at all rigid and by the absence of the prominent midrib which characterizes that species. The pubescence too is longer and more diffuse.

Specimens Examined.—ALBERTA: Tunnel Mt., Banff, May 9, 1902, *N. B. Sanson;* Sulphur Mt., Banff, June 16, 1901, *L. R. Waldron.* WASHINGTON: Yakima Region, 1882, *Brandegee,* no. 371. WYOMING: among rocks on the summit, the Thunderer, Yellowstone Park, July 13, 1899, *A. Nelson & E. Nelson,* no. 5818 (type in Ry. Mt. Herbarium); Mt. Washburn, Yellowstone Park, Aug. 1885, *Tweedy,* no. 566.

10. D. BELLII Holm, Repert. Nov. Sp. Fedde 3: 338. 1907.

I have seen one specimen of this species and that is from "crevices of rocks," Mansfield Island, Hudson Bay, Aug. 30, 1884, *Dr. R. Bell.*

11. **Draba vestita** Payson n. sp.

Very densely cespitose perennial; caudex much branched; leaves persistent and densely clothing the branches of the caudex; 5–7 mm. long, 1 mm. or more wide, broadly linear, thin and not at all rigid, midvein evident; pubescence rather long, involved, hairs in large part unbranched, none really stellate; sepals pubescent; petals apparently yellow, about twice as long as sepals: fruiting inflorescence corymbose; pods rather few (4–6), broadly lanceolate, flattened, densely pubescent with simple or branching hairs, 5–8 cm. long: style about 1 mm. long: seeds not winged.

The name *D. Gilbertiana* has been given to herbarium sheets of this species by Dr. Gilg. Unfortunately it has been impossible to discover his type and so, in conformity to the Vienna rules, a new name has been given to the species.

Specimens Examined.—Alberta: Sheep Mt., Waterton Lake, July 28–31, 1895, *Macoun*, no. 10278. British Columbia: Heights above Carbonate Draw, Beaverfoot Mts., July 13, 1904, *R. T. Shaw*, no. 305. California: Tiukuk Knob, Placer Co., Aug. 12, 1892, *C. F. Sonnee*, no. 15; summit of range between Devil's Cliff and Linker's Knob, Aug. 10, 1901, *Kennedy & Doten.* Montana: Bridger Mts., June 15, 1897, *Rydberg & Bessey*, no. 4173; Upper Marias Pass, Aug. 3, 1883, *Canby*, no. 28 (type in Ry. Mt. Herbarium); Mt. Bridger, June 26, 1899, *Blankinship.* Oregon: cliffs of the Wallowa Mts., July 31, 1899, *Cusick*, no. 2307. Washington: Cascade Mts., 1882, *Brandegee*, no. 373. Wyoming: Yellowstone Park, 1884, *Tweedy*, no. 204.

12. D. nivalis Lilj. Svensk. Vet. Akad. Handl. **1793**: 208. 1793.

Specimens Examined.—Alaska: U. S. Coast Survey, 1871–72, *M. W. Harrington.* Colorado: Elk Mts., 1881, *Brandegee*, no. 13268; Sawatch Range, *Brandegee*, no. 12714 (the Colorado specimens seen are not typical). Greenland: Distr. Holssenborg, Aug. 2, 1886, *L. Ko.derup Rosenvinge.* Hudson Strait: Nottingham Island, Aug. 24, 1884, *R. Bell.* Labrador: Dead Islands, Aug. 17, 1882, *J. A. Allen;* Okkak, N. E. Labrador, *G. Auspach;* northern Labrador, 1873, *G. Auspach*, no. 404.

13. D. lonchocarpa Rydb. Mem. N. Y. Bot. Gard. **1**: 181. 1900.
D. nivalis elongata Wats. Proc. Amer. Acad. **23**: 258. 1886.

Specimens Examined.—Canada: Kicking Horse River, Ry. Mts., Aug. 13, 1890, *Macoun;* Kicking Horse Lake, Ry. Mts., Aug. 12, 1890. *Macoun.* Idaho: rock crevices, Parker Mt., Custer Co., July 17, 1916, *Macbride & Payson*, no. 3240. Montana: Boulder Creek, Aug. 1887, *Tweedy*, no. 36; Upper Marias Pass, Aug. 3, 1883, *Canby*, no. 26; McDonald's Peak, Mission Range, July 19, 1883, *Canby*, no. 27. Washington: Mt. Paddo, July 12, 1886, *Suksdorf*, no. 836.

14. **Draba laevicapsula** Payson n. sp.

Loosely cespitose perennial; caudex branching, leafy branches occasionally 1–2 cm. long: leaves linear, 7–10 mm. long, narrowed slightly at base, usually obtuse, not rigid, midvein evident; pubescence rather loosely stellate, marginal cilia evident, especially toward base of leaves: scapes slender, glabrous, about 5 cm. long, naked or with one or two small bracts: flowers unknown: fruiting inflorescence racemose, comparatively short (about 2 cm.): pods 4–7, narrowly lanceolate, flattened, 7–9 mm. long, 2–3 mm. wide: style scarcely 1 mm. long: seeds not winged.

Dr. Gilg apparently confused *D. laevicapsula* with *D. incerta* but it seems rather to be associated with *D. oligosperma.*

Specimens Examined.—CANADA: Rocky Mts., Aug. 1885, *Macoun;* IDAHO: summit of Steven's Peak, Coeur D'Alene Mts., Aug. 5, 1895, *Leiberg,* no. 1477 (type in Ry. Mt. Herbarium). MONTANA: Upper Marias Pass, Aug. 3, 1883, *Canby,* no. 29.

15. **Draba cyclomorpha** Payson n. sp.

Cespitose perennial: leaves clustered on the apices of the many branches of the caudex, rounded obovate, fleshy, midnerve indistinct, 5–6 mm. long, 3–4 mm. broad; pubescence simple, largely confined to the leaf margins: scapes leafless, sparingly pilose, 1–2 cm. long: flowers unknown: fruiting raceme short and corymbose: pods 3–10, glabrous, flattened, typically nearly circular but at times oblong, 4–5 mm. broad: style scarcely 1 mm. long: seeds not winged.

This species has been confused with *D. Lemmoni* to which it is in fact most nearly related. It differs from that species principally in the glabrous, broader pod.

Specimens Examined.—OREGON: Alpine Wallowa Mts., Aug. 29, 1900, *Cusick,* no. 2497 (type in Ry. Mt. Herbarium); alpine summits, Powder River Mts., Aug., 1886, *Cusick,* no. 1344.

16. D. LEMMONI Wats., Bot. Calif. II. 430. 1880.

Specimens Examined.—CALIFORNIA: summit of Mt. Lyell, Aug. 19, 1878, *Lemmon* (co-type); Mt. Dana, July, 1902, *Hall & Babcock,* no. 3606; Mt. Dana, June 28, 1863, *Brewer,* no. 1735; eastern base of Mt. Brewer, July 4, 1864, *Brewer,* no. 2811; Mt. Goddard, July, 1900, *Hall & Chandler,* no. 668; Little Kern Cr., 1897, *Purpus,* no. 5118; Mt. Dana, July, 1901, *H. M. Evans.*

17. **Draba asterophora** Payson n. sp.

Loosely cespitose perennial with rather long trailing caudex-branches: leaves about 1 cm. long, 4–5 mm. wide, obovate to oblanceolate, obtuse, thickish, midvein obscure; pubescence rather sparse, consisting mostly of long stalked, cruciform hairs, simple cilia almost entirely wanting; scapes slender, glabrous, 3–4 cm. long; fruiting inflorescence shortened with a tendency to become corymbose; pods 6–10, broadly lanceolate, 6–8 mm. long, 4 mm. broad, flattened, glabrous; style short (about .5 mm. long) but evident: seeds flattened, broadly winged.

D. asterophora is evidently allied to *D. Lemmoni* and *D. cyclomorpha* as shown by the similar leaves and fruiting racemes. The ranges of these three species also are rather close. The stellate hairs and winged seed make *D. asterophora* easily separable. But one specimen has been seen and that is from an altitude of 9,000 ft. on Mt. Rose, Washoe County, Nevada, Aug. 17, 1905, *P. B. Kennedy*, no. 1154 (type in Ry. Mt. Herbarium).

18. **Draba Mulfordae** Payson n. sp.

Cespitose perennial: leaves linear or slightly spatulate, obtuse, 7–10 mm. long, 1–2 mm. broad, not rigid, midvein obscure often rising above the ground on leafy shoots or sobols; pubescence of strong marginal cilia and long stalked stellate or branching hairs; scapes slender, pubescent, 3–4 cm. long; sepals pubescent, petals white (?), about three times as long as the sepals; fruiting inflorescence elongated; pods 8–12, lanceolate, 6–8 mm. long, flattened, pubescent; style slender, over 1 mm. long; seeds not winged.

This plant is rather intermediate between the *oligosperma* and the *ventosa* group. The linear leaves, strong cilia and elongated raceme ally it to the former and the tendency to produce leafy shoots to the latter. But one specimen has been seen; it is from Soda Springs, Idaho, June 21, 1892, and was collected by *A. Isabel Mulford* (type in Missouri Botanical Garden Herbarium).

19. D. VENTOSA Gray, Amer. Nat. **8**: 212. 1874.

D. Howellii Watson, Proc. Amer. Acad. **20**: 354. 1885.

D. sobolifera Rydb. Bull. Torrey Club, **30**: 251. 1903.

This seems to be the most variable species of the group. A number of varieties might be made separating plants of different localities but it seems to be impossible to draw specific lines within the group of specimens cited. It should be noted here that the style in *D. sobolifera* is .5 mm. long instead of 5 mm. as the description reads.

Specimens Examined.—CALIFORNIA: Siskiyou Mts., June 16, 1884, *Howell* (type no. of *D. Howellii*). NEVADA: Schellbourne, July 11, 1891, *Jones*. OREGON: head of Divine Creek, Steins Mts., June 14, 1901, *Cusick*, no. 2569; Steins Mts., June 2, 1885, *Howell*. UTAH:

Tate Mine, Marysvale, Aug. 28, 1894, *Jones*, no. 5936 (type no. of *D. sobolifera* Rydb.); Delano Peak, July 26, 1905, *Rydberg & Carlton*, no. 7231; near Beaver, June 7, 1913, *H. Redeker*, no. 50. WYOMING: High Peak between Snake River and Wind River Valleys, 1873, *Parry* (type no. of *D. ventosa*).

20. **Draba cruciata** Payson n. sp.

Cespitose perennial; caudex-branches slender; leaves oblanceolate, usually toothed, thickish, midnerve obscure, 7–10 mm. long, 2–3 mm. broad, acute or acutish; pubescence stellate, each hair usually bearing four arms, ciliae wanting or inconspicuous; scapes slender, 5–7 cm. long: sepals yellow, glabrous or pubescent; petals yellow, about three times as long as sepals: fruiting raceme elongated: pods narrowly lanceolate, 7–9 mm. long, 2 mm. broad, glabrous or simply pubescent, flattened: style slender, 1 mm. long; seeds not winged.

The relationship of this plant is not at all evident. Hall suggests that it is near *D. Lemmoni* and if it is, it should be placed with *D. asterophora* on account of the branched cruciform pubescence. The slightly toothed leaves are a most interesting development. CALIFORNIA: Vicinity of Mineral King, Tulare Co., July 10, 1904, *Hall & Babcock*, no. 5361 (type in Ry. Mt. Herbarium).

21. D. UNCINALIS Rydb. Bull. Torrey Club **30**: 251. 1903.

I have seen no specimens of this species.

Type Locality.—Tate Mine, Marysvale, Utah.

22. **Draba sphaeroides** Payson n. sp.

Loosely cespitose perennial; caudex much branched: leaves clustered on the apices of the caudex-branches, narrowly spatulate, obtuse, green, 3–5 mm. long; pubescence rather long, ciliate and branching, but few truly stellate hairs present: scapes naked, slender, sparingly pubescent with branched or stellate hairs, 1–1.5 cm. long: sepals glabrous or sparingly pubescent; petals yellow, twice as long as the sepals: fruiting inflorescence racemose, 1.5–2 cm. long: pods 6–12, glabrous, ovoid, scarcely flattened, 3–4 mm. long; style slender, about 1 mm. long.

Plants with globose pods are more or less anomalous in this genus but in aspect and all other characters this plant is so obviously a *Draba* that no one would think of placing it elsewhere. Its affinities are doubtless with *D. oligosperma*. Collected above receding snow at an altitude of 10,800 ft. on Jarbidge Peak, Nevada, July 8, 1912, *Nelson & Macbride*, no. 1981 (type in Ry. Mt. Herbarium).

23. **Draba pterosperma** Payson n. sp.

Loosely cespitose perennial; caudex branched; leaves mostly in round tufts either on the apices of the caudex-branches or rising above the ground on sparingly leafy shoots or sobols, oblong, 3–5 mm. long, 1–2 mm. broad, rounded at the apex, midvein evident; pubescence silvery, loosely stellate, cilia present: scapes slender, pubescent, 2–6 cm. long: flowers showy; sepals pubescent; petals yellow, 7–8 mm. long, over twice as long as the sepals: fruiting inflorescence elongated: pods 6 or 8, broadly lanceolate, 8–9 mm. long, 4–6 mm. broad, flattened and often unsymmetrical, pubescent with stellate hairs: style slender, 2 mm. or more long: seeds about 4 in each cell, broadly winged.

Because of the winged seeds in this species and in *D. asterophora* one would be inclined to consider them closely related but such is probably not the case. This plant seems to be related to *ventosa* and so we must assume that the development of winged seeds has been accomplished independently by two different groups.

Specimens Examined.—CALIFORNIA: rock crevices, Marble Mt., Siskiyou Co., July 10, 1910, *Geo. D. Butler*, no. 1716 (type in Ry. Mt. Herbarium); Marble Mt., June, 1901, *H. P. Chandler*, no. 1654 (Mo. Bot. Gard. Herbarium and perfectly typical).

24. D. ARGYREA Rydb. Bull. Torrey Club **30**: 251. 1903.

Type Locality.—Sawtooth Mts., Idaho, head of Pettit Lake.

The specimens cited below are somewhat doubtfully referred here. Since, however, no authentic material of *D. argyrea* has been available, since our plants agree fairly well with the description and are from the same vicinity it has been thought best to leave the question undecided.

Specimens Examined.—IDAHO: rock crevices, alpine basin in Sawtooth Mts., above Redfish Lake, Blaine Co., Aug. 9, 1916, *Macbride & Payson*, no 3677; crevices in granitic rocks, Smoky Mts., Blaine Co., Aug. 13, 1916, *Macbride & Payson*, no. 3734.

25. **Draba sphaerocarpa** Macbride and Payson, n. sp.

Cespitose perennial; caudex much branched; leaves mostly borne in tufts on erect, nearly leafless shoots that rise above the caudex-branches, oblong or obovate, obtuse, 4–7 mm. long, thickish and midvein indistinct; pubescence finely and densely stellate; leaves silvery, cilia absent: scapes pubescent, rather stout: flowers unknown (probably yellow): fruiting raceme elongated, developing almost from very base of scape: pods many (8–12), scarcely compressed or flattened, ovate, stellately pubescent, 2–5 mm. long: style slender, 1 mm. or more long: seeds not winged.

This plant is most closely related to *D. argyrea* and in leaf characters it is practically identical. It is distinguished from that species by the small, subspherical pods and the peculiar inflorescence which develops from near the base of the scape. *D. sphaerocarpa* was collected at a much lower elevation than were the specimens referred to *D. argyrea.*

Type.—IDAHO: dry, granitic washes near the head of Redfish Lake, Blaine Co., Aug. 9, 1916, *Macbride & Payson*, no. 3677*a* (Gray Herbarium).

26. D. SUBSESSILIS Watson, Proc. Am. Acad. **23**: 255. 1888.

Type Locality.—"On the White Mts. of Mono Co., California, at 13,000 ft. altitude" (*W. H. Shockley*, July, 1886).

Specimens Examined.—CALIFORNIA: Mt. Dana, June 28, 1863, *Brewer*, no. 1735*a*; White Mts., Mono Co., Aug., 1885, *W. H. Shockley.*

UNIVERSITY OF WYOMING,
LARAMIE

THE OSMOTIC CONCENTRATION OF THE TISSUE FLUIDS OF JAMAICAN MONTANE RAIN-FOREST VEGETATION[1]

J. Arthur Harris and John V. Lawrence

I. Introductory Remarks

Purpose of Investigation.—This paper is one of a series in which various problems involving the investigation of the osmotic pressure or osmotic concentration of the fluids of plant tissues are treated. Specifically it presents an extensive series of determinations of the freezing-point lowering of the extracted leaf sap of plants from the Blue Mountains of Jamaica, discusses the differences in these values in their relation to local differences in the environmental complex, and briefly compares the series as a whole with others now available.

In another place (Harris, Lawrence and Gortner, 1916) we have put forward in detail the arguments for the carrying out of such studies as a regular part of systematic and thoroughgoing phytogeographical investigation. It seems unnecessary, therefore, to repeat these arguments here.

After completing a series of determinations of the osmotic concentration of the tissue fluids of a number of species of plants from the southwestern deserts, in the vicinity of the Desert Laboratory during the winter and spring months of 1914, and comparing them (Harris, Lawrence and Gortner, 1915) with a series made in the more mesophytic habitats in the neighborhood of the Station for Experimental Evolution on Long Island, the next most desirable step seemed to be the investigation of the sap properties of the plants of an extremely hygrophytic region.

Since such field studies could be most conveniently carried out during the winter months, at a time when we could be absent from experiments under way at the Station for Experimental Evolution, it was quite natural to think of the Tropical Laboratory at Cinchona, established by the New York Botanical Garden and later maintained by the British Association for the Advancement of Science and the Jamaican local government, as the most promising locus for such work. This station presents the advantages of furnishing living quarters and laboratory space on the edge of a primaeval montane rain forest within twenty miles of a point where ice, essential for the preliminary freezing of tissues for the extraction of sap, can be obtained. This was quite successfully packed over the Port Royal Mountains, through the Yallahs river valley and up to Cinchona on mule back by negro helpers.

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[1] Results of investigations carried on at Cinchona, by courtesy of the British Association for the Advancement of Science and the Jamaican local government, under the joint auspices of the department of botanical research and the department of experimental evolution of the Carnegie Institution of Washington, and with the collaboration of the New York Botanical Garden.

To Professor Bower and the other members of the British Association committee in charge of the Tropical Laboratory at Cinchona and to Mr. Wm. Harris, F.L.S., superintendent of public gardens and plantations, we are indebted not only for the use of the laboratory but for other courtesies that added to the success and pleasure of our work while in Jamaica.

Characteristics of the Region Investigated.—The higher portions of the Blue Mountains are characterized by a relatively low but uniform temperature, by a large and well-distributed rainfall, accompanied by much fog and cloudiness and high relative humidity.

The rainfall upon the northern is far greater than that upon the southern slopes of the mountains. The averages given by Shreve (1914) for the upper mountains, in which all our collections were made, are:

Cinchona	105.70 inches
New Haven Gap	113.85 inches
Blue Mountain Peak	130.48 inches

Notwithstanding the heavy rainfall there are neither ponds nor constant streams above 4,500 feet, but in places there are depressions on the higher portions of the main ridge of the mountains which are developed as sphagnum bogs. Below 4,500 feet the water emerges to feed numerous swift mountain streams. Transient water courses are found much higher.

While the rainfall is large it is not comparable with the maximum precipitations known in other tropical plant environments. Furthermore the amount varies greatly from year to year, both in quantity and distribution. Thus Shreve (1914), in working through the records which have been kept at Cinchona for the past thirty-nine years, finds variation in the total annual precipitation from about 59 to about 179 inches. In October, the rainfall has varied from about 3 to 43 inches. In February, precipitation has ranged from less than an inch to nearly 13 inches. At New Haven Gap during the three months of April, May and June, 1892, there was not a measurable amount of rainfall, whereas during the same three months in 1894 there fell 62 inches of water.

Thus the vegetation is by no means free from occasional periods of drought.

Notwithstanding this fact, moisture is so great in quantity and so uniform in distribution that it supports a dense evergreen arborescent and herbaceous vegetation, a large proportion of the constituent species of which are of a pronouncedly hygrophilous character. As a factor in the development and maintenance of the vegetation, the distribution as well as the actual quantity of the precipitation is a factor of great importance. Precipitation is almost exclusively in the form of light showers of brief duration or gentle and long continued rain, but never in the torrential downpours so characteristic of deserts and tropical lowlands. Transient showers of too brief duration to be registered as giving a measurable quantity of rainfall are frequent. Shreve gives a table showing that at Cinchona on an average from one third to two thirds of the days of the twelve individual months of the year have a measurable precipitation.

On the northern slopes fog is prevalent from below 4,500 feet to the summits of the highest peaks from 10 a. m. to 4 p. m. on a large proportion of the days during all the months of the year, with the possible exceptions of July and August. Fog is much less frequent on the southern exposure of the mountains, but even here it is often seen on the upper slopes, and a large percentage of the days are cloudy or partially cloudy. Shreve, after nearly a year's residence in the Blue Mountains, describes the condition as follows: "The typical course of the day's weather is: clear from sunrise until 9 to 11 a. m., intermittently or entirely cloudy until nearly sunset, with two or three hours of fog in the mid-day or early afternoon, the sun setting clear. Rain usually occurs in the mid-day or early afternoon and the night is clear."

As a consequence of the high and well-distributed rainfall and the prevalence of fog, atmospheric humidity is high, ranging from about 80 to about 89 percent in the various months of the year, with an annual average of about 84 percent.

Temperature is low and remarkably uniform throughout the year. At a depth of six feet at Cinchona the monthly mean soil temperature is 16.4° C., with a mean annual range of 1.5°. For air temperatures the annual mean is 16.0°, the annual mean range 2.9°, and the average daily range 6.6°.

Our work was of necessity carried out within a radius sufficiently narrow to permit of the collections being made afoot, and brought back to the Laboratory for freezing within a few hours. Materials were drawn from the territory made accessible by the trail from Cinchona through Morce's Gap to a point somewhat south of Vinegar Hill, by that from Morce's Gap to John Crow Peak, by that from Cinchona to a point on one of the Green River affluents south of New Haven Gap, and by that from Cinchona through New Haven Gap to the lower slopes of Sir John Peter Grant Peak. Collections were by no means limited to the immediate vicinity of the trails, but were also drawn from the denser parts of the jungle, which was pretty thoroughly penetrated in various directions.

While a few determinations are based upon collections made between 5,500 and 6,000 feet, especially from New Haven Gap and from the slopes and summit of John Crow Peak, the main bulk of our constants are based on samples gathered between 4,500 and 5,500 feet. Below 4,500 feet conditions change rapidly. Thus at Resource, one mile south of Cinchona and 1,300 feet lower (3,700 as compared with 5,000 feet), the mean rainfall is about 68 as compared with about 106 inches per annum at the Laboratory. The fogs which are so characteristic a feature of the northern slopes of the mountains, and which roll over the ridges from the windward sides, are dissipated on the lower leeward (southern) slopes. Thus conditions are not merely warmer but far drier. Here, too, much of the natural vegetation, which in most of the area studied was in a primaeval condition, has been replaced or distinctly modified by agricultural operations—chiefly the planting of Arabian coffee, which thrives and because of the superiority of the product is commercially profitable in a region so broken as to be useful for only the more valuable hand-tilled crops.

Materials and Methods.—In order that the constants of the present study may be comparable with those derived from other regions it has seemed desirable to limit the determinations to those based on terrestrial plants. Epiphytic forms are reserved for treatment, with comparable forms from other regions, in a special publication.

In a habitat in which erosion is so active, epiphytes are frequently brought to the ground by the fall of trees. Furthermore, conditions on the litter-covered forest floor, on large fallen and partially decayed logs, and on the higher limbs of trees, differ by only imperceptible degrees. Thus our separation of the epiphytes from the terrestrial forms has of necessity been somewhat arbitrary.

All of the Bromeliaceae we have omitted from the present treatment.

Of the Orchidaceae we are publishing determinations for the terrestrial *Prescottia stachyoides* and *Stenorrhynchos speciosum*. *Epidendrum verrucosum* we have included since we always collected it growing in soil on rocky banks. Fawcett and Rendle give its occurrence as "on trees, rocks and dry banks." *Epidendrum imbricatum*, which Fawcett and Rendle cite as occurring on trees and which we found growing as a typical epiphyte, we have omitted from the present paper. The parasites have been discussed in an earlier number of this Journal (Harris and Lawrence, 1916).

The species of the genus *Peperomia* have caused considerable trouble. They may be either truly epiphytic, rooted in the masses of leaf mould on fallen logs, or terrestrial in peaty soil. So far as we were able to observe *P. stellata* is always terrestrial. We have therefore included it, but have reserved all other species of *Peperomia* for a special memoir on epiphytic vegetation.

Blakea trinervia and *Tradescantia multiflora*, which may be either rooted in the soil or epiphytic, have been included in this paper.

Methods.—The methods employed were those of previous papers of this series. Considerable difficulty of a purely physical sort was encountered in the collection of the samples. Much of the work had to be carried out in the rain or in tangled vegetation dripping wet from recent rain or fog. It was often necessary, therefore, for one worker to crouch under a poncho and wipe each leaf dry with absorbent tissue before it was placed in the collecting tubes for preliminary freezing (Gortner and Harris, 1914).

The frozen tissue was squeezed with the greatest thoroughness possible in a press with a powerful hand screw to avoid any possibility of the differential extraction of sap as noted by Dixon and Atkins (1913) and ourselves (Gortner, Lawrence and Harris, 1916).

The freezing-point lowering of the filtered sap was determined by means of ether or carbon bisulphide vaporized by a dried air current in a vacuum jacketed bulb.

The results are expressed in terms of freezing-point lowering, Δ, corrected for undercooling, and in atmospheres pressure P from a published table (Harris and Gortner, 1914).

Classification of Habitats.—In these studies it has been our policy to adopt in so far as possible the classification of plant habitats drawn up by specialists in ecology or phytogeography. Such a course makes for simplicity and lack of confusion in the literature, lends added value to such habitat studies as have already been made by correlating with them new kinds of observations, and finally precludes any possibility of bias in the classification of determinations in a way to make them agree with any preconceived theory.

For the Blue Mountain region it has been possible to follow the classification presented in the splendid work of our colleague Forrest Shreve (1914) whose extended experience in the montane region of Jamaica and whose analyses of the previous scattered literature and meteorological data have made it unnecessary for us to go back of his large publication on the region.

For descriptive details presented in a most readable manner and a wealth of carefully selected illustrations the reader must turn to Shreve's book. Here only the most salient and essential points will be set forth.

The fundamental division is that into the two main slopes of the mountain chain. These are designated as windward and leeward rather than northern and southern to emphasize the predominant influence of the moisture-laden trade winds in determining the characteristics of the vegetation. The subdivision of the two main slopes is made on the basis of topography, into ravines, slopes and ridges. In carrying out our work we have found it desirable to emphasize certain of these regions at the expense of others. Such descriptive details as are essential will be given under the discussions of the individual habitats.

We have not found it practicable to consider individually all of the five types of habitats recognized by Shreve.

Because of the morphologically xerophilous character of its scrub vegetation we desired to investigate rather fully the sap properties of the "ruinate" of the once cleared southern slopes. This seemed to us more important than a consideration of the primaeval forest of the leeward slopes. As the other extreme in the vegetation of the southern side of the ridge, the vegetation of the leeward ravine seemed desirable.

In dealing with the collections from the windward sides of the mountains we have not found it practicable to follow the treatment accorded them by Shreve who discusses the ravines and the slopes separately. The two habitats blend quite imperceptibly into each other. The distinction between the vegetation of the two has seemed to us to be primarily one of the loftiness of the trees and the abundance of the extremely hygrophilous ferns, mosses, and hepatics. While an investigation of the concentration of the sap in the bryophytes and filmy ferns that are so characteristic a feature of the more hygrophytic habitats would be of great interest, we preferred to devote our time to the study of arborescent and herbaceous seed plants of the type to be met with in other regions with which comparisons are to be drawn.

For this reason we have treated the collections from the leeward ravines and leeward slopes together.

Our collections have, therefore, been distributed among the following habitats.

I. Ruinate of the Leeward Slopes.
II. Leeward Ravines.
III. Ridges.
IV. Windward Slopes and Ravines.

The distinction between these habitats is by no means always sharply marked. Ravines and ridges are merely the extremes of the topographic series. Between them and the intervening slopes there is, from the purely topographical side, no sharp line of demarcation.

Furthermore, the habitat distinctions are not based primarily upon the substratum but upon meteorological conditions. Air movements undoubtedly play a considerable rôle in determining the character of the vegetation. Thus fog is often blown over the main ridge, rolling down the leeward slopes for some distance, to be dissipated below. The vegetation of the ridges which are at the same time gaps exhibits many of the characteristics of the ravine.

In view of these facts it is altogether improbable that any two botanists would agree exactly upon the classification into habitats of a series of 398 collections—the number upon which the present discussion is based. While in some cases our disposition of a given determination may have been somewhat arbitrary, it was not influenced in any measure by the magnitude of the constant, for the collections were all classified before the corrected freezing point lowerings were calculated. Thus there seems no possibility of personal equation influencing the results.

II. Presentation of Data

I. *Ruinate of Leeward Slopes*

The slopes which were once cleared for coffee or cinchona planting but have since been abandoned—in a large part, long ago—are known locally as ruinate.

The ruinate is characterized, as is of course to be expected, by a relatively large number of introduced, in some cases widespread, species.

While the ruinate has been described by writers as a xerophilous scrub formation, it occupies an area supplied with an abundance of precipitation, quite as much in fact as the primaeval forest of the same slopes.

In so far as the conditions are really those of a xerophytic environment they must be due to (*a*) edaphic conditions influencing water absorption, and (*b*) to the lowness and openness of the stand, permitting free air movements with consequent increased transpiration.

The classification of this vegetation as xerophilous is due, we believe, to two factors. First, in contrast to the extreme hygrophily of the ravines of both leeward and windward slopes, the structurally really mesophytic species of the ruinate have a far more xerophytic aspect than they would if growing in a region of more moderate humidity, just as they would pass for decidedly mesophytic types in deserts like those of southern Arizona. Second, there are a number of truly desert species which have a profound effect upon the physiognomy of the vegetation. Agave is not common but *Yucca aloifolia* is frequently seen. *Baccharis scoparia* is probably the chief form lending a xerophytic aspect to the vegetation.

What we have just said concerning the ruinate applies to only the areas in the neighborhood of 5,000 feet where our determinations were made. Below this level, and especially on the southern face of the Port Royal mountains, conditions are much drier and the truly desert species more numerous.

A habitat in which such introduced forms as *Daucus Carota*, *Pastinaca sativa* and *Plantago lanceolata* thrive, and in which occurs a number of species common to this and one or more of the hygrophytic habitats, can hardly be regarded as truly xerophytic.

The determinations from the ruinate are given in the accompanying protocol.

Since the data are presented in a uniform way for the four habitats, an explanation of the form of these lists may be given here.

The plants are first of all divided into ligneous and herbaceous. Under each of these groups the species are, for convenience of reference, arranged alphabetically. The values of Δ and P opposite the species names are averages whenever more than a single determination for the species could be secured in the habitat. In such cases the values are designated by bars, $\bar{\Delta}$ and $\bar{P}$, the individual determinations upon which these averages are based with their dates of collection are given beneath the species name and its average constants for the habitat in question. In cases in which only a single determination could be secured, the values of Δ and P are given, with the date of collection, in place of the average value.

Ligneous Plants

Asclepias physocarpa (E. Meyer) Schlecht. Feb. 28, $\Delta = 0.86$, $P = 10.4$

Baccharis scoparia (L.) Sw. $\bar{\Delta} = 1.18$, $\bar{P} = 14.2$

Feb. 6, $\Delta = 1.10$, $P = 13.3$; Feb. 18, $\Delta = 1.15$, $P = 13.8$; Feb. 24, $\Delta = 1.28$, $P = 15.4$.

Bidens incisa (Ker.) G. Don Feb. 7, $\Delta = 0.91$, $P = 11.0$

Bocconia frutescens L. $\bar{\Delta} = 0.91$, $\bar{P} = 11.0$

Feb. 5, $\Delta = 0.82$, $P = 9.9$; Feb. 28, $\Delta = 0.99$, $P = 12.0$.

Borreria verticillata (L.) Meyer Feb. 5, $\Delta = 0.68$, $P = 8.2$

Caesalpinia sepiaria Roxb. $\bar{\Delta} = 0.97$, $\bar{P} = 11.7$

Mar. 6, $\Delta = 0.95$, $P = 11.5$; Mar. 6, $\Delta = 0.98$, $P = 11.8$.

Cestrum odontospermum Jacq. Mar. 6, $\Delta = 0.99$, $P = 11.9$

Citharexylum caudatum L. Mar. 18, $\Delta = 2.03$, $P = 24.4$

Coffea arabica L. Mar. 6, $\Delta = 1.29$, $P = 15.5$

Cracca grandiflora (Vahl.) Kuntze Feb. 5, $\Delta = 0.85$, $P = 10.3$

Crotalaria Saltiana Andr. Feb. 8, $\Delta = 0.82$, $P = 9.9$

Dodonaea jamaicensis DC. $\Delta = 1.18$, $\bar{P} = 14.2$

Feb. 5, $\Delta = 1.05$, $P = 12.7$; Feb. 7, $\Delta = 1.09$, $P = 13.1$; Feb. 26, $\Delta = 1.41$, $P = 16.9$.

Duranta repens L. $\bar{\Delta} = 1.26$, $\bar{P} = 15.2$

Feb. 28, $\Delta = 1.29$, $P = 15.5$; Feb. 28, $\Delta = 1.25$, $P = 15.0$; Mar. 6, $\Delta = 1.25$, $P = 15.1$.

Echites torosa Jacq. Feb. 5, $\Delta = 1.08$, $P = 13.0$

Eroteum theoides Sw. (Cleyera theoides (Sw.) Choisy) $\overline{\Delta} = 1.14$, $\overline{P} = 13.8$

Feb. 14, $\Delta = 1.03$, $P = 12.4$; Feb. 28, $\Delta = 1.15$, $P = 13.9$; Mar. 6, $\Delta = 1.19$; $P = 14.3$; Mar. 6, $\Delta = 1.20$, $P = 14.5$.

Young leaves were also taken Feb. 14 and gave: $\Delta = 1.27$, $P = 15.3$.

Eupatorium glandulosum H. B. K. $\overline{\Delta} = 0.82$, $\overline{P} = 9.8$

Feb. 28, $\Delta = 0.82$, $P = 9.8$; Mar. 6, $\Delta = 0.81$, $P = 9.8$.

Eupatorium heteroclinium Griseb. $\overline{\Delta} = 1.10$, $\overline{P} = 13.2$

Feb. 5, $\Delta = 1.17$, $P = 14.0$; Feb. 7, $\Delta = 1.03$, $P = 12.4$.

Eupatorium triste DC. Feb. 5, $\Delta = 1.08$, $P = 13.0$

Garrya Fadyenii Hook. $\overline{\Delta} = 2.13$, $\overline{P} = 25.6$

Feb. 7, $\Delta = 1.92$, $P = 23.0$; Feb. 14, $\Delta = 2.34$, $P = 28.1$.

Iresine paniculata (L.) Kuntze Feb. 5, $\Delta = 0.85$, $P = 10.3$

Lantana Camara L. $\overline{\Delta} = 0.81$, $\overline{P} = 9.8$

Feb. 5, $\Delta = 0.74$, $P = 9.0$; Feb. 14, $\Delta = 0.94$, $P = 11.3$; Mar. 6, $\Delta = 0.76$, $P = 9.2$.

Lantana reticulata Pers. Feb. 28, $\Delta = 0.76$, $P = 9.2$

Lantana stricta Sw. $\overline{\Delta} = 0.73$, $\overline{P} = 8.8$

Feb. 5, $\Delta = 0.66$, $P = 8.0$; Feb. 14, $\Delta = 0.76$, $P = 9.1$; Mar. 6, $\Delta = 0.77$, $P = 9.2$.

Mecranium virgatum (Sw.) Triana Feb. 28, $\Delta = 0.71$, $P = 8.6$

Miconia quadrangularis (Sw.) Naud. Mar. 6, $\Delta = 0.91$, $P = 10.9$

Micromeria obovata Benth. Feb. 7, $\Delta = 0.76$, $P = 9.1$

Oreopanax capitatum (Jacq.) Dec. & Pl. $\overline{\Delta} = 1.59$, $\overline{P} = 19.1$

Feb. 14, $\Delta = 1.66$, $P = 19.9$; Feb. 28, $\Delta = 1.59$, $P = 19.1$; Mar. 18, $\Delta = 1.53$, $P = 18.3$.

Young leaves taken with the sample of March 18 gave: $\Delta = 1.14$, $P = 13.8$

Passiflora edulis Sims Mar. 18, $\Delta = 1.58$, $P = 19.0$

Phenax hirtus (Sw.) Wedd. Feb. 28, $\Delta = 0.76$, $P = 9.2$

Psychotria corymbosa Sw. Feb. 14, $\Delta = 0.82$, $P = 9.9$

Quercus sp. Mar. 19, $\Delta = 1.10$, $P = 13.3$

Rapanea ferruginea (R. & P.) Mez Feb. 14, $\Delta = 1.18$, $P = 14.2$

Relbunium hypocarpium (L.) Hemsl. Feb. 5, $\Delta = 0.76$, $P = 9.2$

Rosa laevigata Michx. Feb. 8, $\Delta = 1.50$, $P = 18.0$

Smilax celastroides Kunth Feb. 14, $\Delta = 1.38$, $P = 16.6$

Young leaves gave: $\Delta = 1.14$, $P = 13.7$.

Triumfetta semitriloba Jacq. Feb. 8, $\Delta = 0.73$, $P = 8.8$

Vaccinium meridionale Sw. $\overline{\Delta} = 1.25$, $\overline{P} = 15.1$

Feb. 7, $\Delta = 1.17$, $P = 14.1$; Feb. 18, $\Delta = 1.25$, $P = 15.1$; Feb. 24, $\Delta = 1.34$, $P = 16.1$.

Vernonia divaricata Sw. $\overline{\Delta} = 1.14$, $\overline{P} = 13.7$

Feb. 14, $\Delta = 1.21$, $P = 14.6$; Feb. 28, $\Delta = 1.17$, $P = 14.0$; Mar. 6, $\Delta = 1.03$, $P = 12.4$.

Viburnum alpinum Macf. $\overline{\Delta} = 1.36$, $\overline{P} = 16.4$

Feb. 5, $\Delta = 1.29$, $P = 15.5$; Feb. 28, $\Delta = 1.42$, $P = 17.1$; Mar. 6, $\Delta = 1.37$, $P = 16.5$.

Yucca aloifolia L. $\overline{\Delta} = 1.63$, $\overline{P} = 16.3$

Feb. 6, $\Delta = 1.78$, $P = 21.4$; Feb. 28, $\Delta = 1.45$, $P = 7.4$; Mar. 6, $\Delta = 1.66$, $P = 20.0$.

The values given are those for the fully matured leaves. A determination from the yellowish leaves which were past their period of maximum physiological activity was taken on Feb. 6 and gave: $\Delta = 1.55$, $P = 18.7$. In the collection of Feb. 28 the young leaves gave $\Delta = 0.79$, $P = 9.5$. Juice extracted from the axis of the plants from which the collection of Feb. 6 was made gave $\Delta = 0.96$, $P = 11.6$.

Herbaceous Plants

Ambrosia peruviana Willd. Feb. 28, $\Delta = 1.02$, $P = 12.2$

Aristea compressa Buch. Feb. 5, $\Delta = 0.72$, $P = 8.7$

Begonia obliqua L. $\overline{\Delta} = 0.36$, $\overline{P} = 4.3$

Feb. 8, $\Delta = 0.35$, $P = 4.2$; Feb. 14, $\Delta = 0.36$, $P = 4.3$; Feb. 28, $\Delta = 0.30$, $P = 3.7$; Mar. 6, $\Delta = 0.41$, $P = 5.0$.

Bidens pilosa L. Feb. 7, $\Delta = 0.74$, $P = 8.9$

Bryophyllum pinnatum (Lam.) Kurz. $\overline{\Delta} = 0.40$, $\overline{P} = 4.7$

Feb. 5, $\Delta = 0.38$, $P = 4.5$; Mar. 6, $\Delta = 0.41$, $P = 4.9$.

Cionosicys pomiformis (Macf.) Griseb. Mar. 18, $\Delta = 1.06$, $P = 12.8$

Daucus Carota L. $\overline{\Delta} = 1.16$, $\overline{P} = 14.0$

Feb. 5, $\Delta = 1.01$, $P = 12.1$; Mar. 6, $\Delta = 1.31$, $P = 15.8$.

Epidendrum verrucosum Sw. $\overline{\Delta} = 0.55$, $\overline{P} = 6.6$

Feb. 6, $\Delta = 0.58$, $P = 7.0$; Mar. 6, $\Delta = 0.55$, $P = 6.6$; Mar. 16, $\Delta = 0.51$, $P = 6.2$.

Hedychium flavum Roxb. × *Hedychium Gardnerianum* Rosc. (?) Feb. 6, $\Delta = 0.67$, $P = 8.1$

Lycopodium clavatum L. $\overline{\Delta} = 0.79$, $\overline{P} = 9.5$

Feb. 18, $\Delta = 0.80$, $P = 9.6$; Feb. 24, $\Delta = 0.78$, $P = 9.4$.

Lycopodium Fawcettii Lloyd & Underw. $\overline{\Delta} = 0.89$, $\overline{P} = 10.6$

Feb. 18, $\Delta = 0.87$, $P = 10.4$; Feb. 24, $\Delta = 0.90$, $P = 10.8$.

Maurandia erubescens (Zucc.) A. Gray. $\overline{\Delta} = 0.84$, $\overline{P} = 10.1$

Feb. 5, $\Delta = 0.86$, $P = 10.3$; Feb. 14, $\Delta = 0.80$, $P = 9.7$; Feb. 28, $\Delta = 0.89$, $P = 10.7$; Mar. 6, $\Delta = 0.79$, $P = 9.5$.

Meibornia uncinata (Jacq.) Kuntze (?) Feb. 8, $\Delta = 0.62$, $P = 7.5$

Pastinaca sativa L. $\overline{\Delta} = 1.27$, $\overline{P} = 15.3$

Feb. 14, $\Delta = 1.35$, $P = 16.3$; Mar. 6, $\Delta = 1.18$, $P = 14.2$.

Pilea grandifolia (L.) Blume (?) Feb. 28, $\Delta = 0.66$, $P = 8.0$

Plantago lanceolata L. $\overline{\Delta} = 1.12$, $\overline{P} = 13.5$

Feb. 8, $\Delta = 0.95$, $P = 11.5$; Feb. 28, $\Delta = 1.13$, $P = 13.6$; Mar. 6, $\Delta = 1.29$, $P = 15.5$.

Verbena bonariensis L. Feb. 8, $\Delta = 0.94$, $P = 11.3$

II. *The Leeward Ravines*

The ravines, like the slopes, of the leeward side of the mountains receive a lighter rainfall, much less fog, and reciprocally more hours of sunshine, than the windward habitats.

The ravines of the leeward slopes are physiographically similar to those of the windward slopes. Both exhibit a forest covering of irregular canopy of larger trees with rich undergrowth of shrubs. The conspicuous difference between the two is chiefly found in the relative scarcity of epiphytes, both Orchidaceae and Bromeliaceae, and particularly of the most hygrophilous of the pteridophytes and the practical absence of tree ferns in the leeward ravines.

Ligneous Plants

Acalypha virgata L. $\bar{\Delta} = 0.87$, $\bar{P} = 10.5$

Feb. 11, $\Delta = 0.78$, $P = 9.4$; Mar. 11, $\Delta = 0.85$, $P = 10.2$; Mar. 18, $\Delta = 0.98$, $P = 11.8$.

Acnistus arborescens (L.) Schlecht. Mar. 11, $\Delta = 0.85$, $P = 10.2$

Besleria lutea L. $\bar{\Delta} = 0.74$, $\bar{P} = 8.8$

Feb. 11, $\Delta = 0.65$, $P = 7.8$; Feb. 26, $\Delta = 0.69$, $P \doteq 8.3$; Mar. 11, $\Delta = 0.85$, $P = 10.2$; Mar. 18, $\Delta = 0.75$, $P = 9.0$.

Bocconia frutescens L. $\bar{\Delta} = 0.79$, $\bar{P} = 9.5$

Feb. 11, $\Delta = 0.75$, $P = 9.0$; Mar. 11, $\Delta = 0.83$, $P = 10.0$.

Young leaves taken in the collection of Feb. 11 gave values only slightly lower than those from mature organs, i. e., $\Delta = 0.72$, $P = 8.6$.

Boehmeria caudata Sw. Mar. 11, $\Delta = 0.86$, $P = 10.3$

Brunfelsia jamaicensis Griseb. $\bar{\Delta} = 0.79$, $\bar{P} = 9.4$

Feb. 11, $\Delta = 0.78$, $P = 9.3$; Mar. 18, $\Delta = 0.79$, $P = 9.5$.

Cestrum hirtum Sw. $\bar{\Delta} = 0.73$, $\bar{P} = 8.8$

Feb. 11, $\Delta = 0.74$, $P = 8.9$; Mar. 18, $\Delta = 0.72$, $P = 8.7$.

Cinchona Sp. $\bar{\Delta} = 0.92$, $\bar{P} = 11.1$

Feb. 11, $\Delta = 0.97$, $P = 11.6$; Mar. 18, $\Delta = 0.87$, $P = 10.5$.

Clibadium terebinthinaceum (Sw.) DC. $\bar{\Delta} = 0.82$, $\bar{P} = 9.9$

Feb. 11, $\Delta = 0.72$, $P = 8.6$; Feb. 26, $\Delta = 0.89$, $P = 10.7$; Mar. 11, $\Delta = 0.86$, $P = 10.4$.

Dendropanax arboreum (L.) Dec. & Pl. $\bar{\Delta} = 1.11$, $\bar{P} = 13.3$

Feb. 11, $\Delta = 1.11$, $P = 13.3$; Mar. 18, $\Delta = 1.10$, $P = 13.3$.

Duranta repens L. $\bar{\Delta} = 1.33$, $\bar{P} = 16.0$

Feb. 11, $\Delta = 1.20$, $P = 14.4$; Mar. 18, $\Delta = 1.45$, $P = 17.5$.

Eupatorium glandulosum H. B. K. $\bar{\Delta} = 0.64$, $\bar{P} = 7.7$

Feb. 26, $\Delta = 0.64$, $P = 7.7$; Mar. 11, $\Delta = 0.64$, $P = 7.6$.

Eupatorium riparium Regel $\bar{\Delta} = 0.78$, $\bar{P} = 9.3$

Mar. 11, $\Delta = 0.77$, $P = 9.2$; Mar. 18, $\Delta = 0.78$, $P = 9.4$.

Fuchsia corymbiflora R. & P. $\bar{\Delta} = 0.67$, $\bar{P} = 8.1$

Mar. 11, $\Delta = 0.73$, $P = 8.8$; Mar. 11, $\Delta = 0.70$, $P = 8.5$; Mar. 18, $\Delta = 0.57$, $P = 6.9$.

Garrya Fadyenii Hook. Feb. 26, $\Delta = 2.23$, $P = 26.8$

Gesneria alpina Urban Mar. 11, $\Delta = 0.52$, $P = 6.3$

Guarea Swartzii DC. $\bar{\Delta} = 0.90$, $\bar{P} = 10.9$

Mar. 11, $\Delta = 0.79$, $P = 9.6$; Mar. 18, $\Delta = 1.02$, $P = 12.3$; Mar. 18, $\Delta = 0.90$, $P = 10.8$.

Hedyosmum nutans Sw. Feb. 26, $\Delta = 0.73$, $P = 8.8$

Iresine paniculata (L.) Kuntze $\bar{\Delta} = 0.96$, $\bar{P} = 11.6$

Feb. 11, $\Delta = 0.89$, $P = 10.8$; Feb. 26, $\Delta = 0.97$, $P = 11.7$; Mar. 11, $\Delta = 1.03$, $P = 12.4$.

Lantana Camara L. $\bar{\Delta} = 0.69$, $\bar{P} = 8.2$

Feb. 11, $\Delta = 0.64$, $P = 7.6$; Feb. 26, $\Delta = 0.73$, $P = 8.8$; Mar. 11, $\Delta = 0.68$, $P = 8.2$; Mar. 18, $\Delta = 0.69$, $P = 8.3$.

Phenax hirtus (Sw.) Wedd. $\bar{\Delta} = 0.77$, $\bar{P} = 9.2$

Feb. 26, $\Delta = 0.74$, $P = 8.9$; Mar. 11, $\Delta = 0.79$, $P = 9.5$.

Phoebe montana (Sw.) Griseb. Feb. 11, $\Delta = 1.02$, $P = 12.3$

Pilea Weddellii Fawc. & Rendle Feb. 26, $\Delta = 0.71$, $P = 8.5$

Piper hispidum Sw. Mar. 18, $\Delta = 0.67$, $P = 8.0$

Psychotria corymbosa Sw. $\bar{\Delta} = 0.68$, $\bar{P} = 8.1$

Feb. 11, $\Delta = 0.66$, $P = 7.9$; Feb. 26, $\Delta = 0.69$, $P = 8.3$.

Rapanea ferruginea (R. & P.) Mez Feb. 26, $\Delta = 1.10$, $P = 13.2$

Young leaves gave: $\Delta = 1.09$, $P = 13.1$.

Rubus jamaicensis Sw. Mar. 18, $\Delta = 1.33$, $P = 16.0$

Senecio Swartzii DC. Mar. 18, $\Delta = 0.66$, $P = 7.9$

Solandra grandiflora Sw. $\bar{\Delta} = 0.82$, $\bar{P} = 9.8$

Mar. 11, $\Delta = 0.79$, $P = 9.5$; Mar. 18, $\Delta = 0.84$, $P = 10.1$.

Tovaria pendula R. & P. Mar. 11, $\Delta = 0.94$, $P = 11.3$

Turpinia occidentalis (Sw.) G. Don Mar. 18, $\Delta = 1.06$, $P = 12.8$

Viburnum villosum Sw. Mar. 18, $\Delta = 1.15$, $P = 13.9$

Herbaceous Plants

Anthurium scandens (Aubl.) Engler $\bar{\Delta} = 0.52$, $\bar{P} = 6.3$

Feb. 11, $\Delta = 0.51$, $P = 6.1$; Mar. 11, $\Delta = 0.53$, $P = 6.4$.

Begonia obliqua L. $\bar{\Delta} = 0.37$, $\bar{P} = 4.5$

Feb. 26, $\Delta = 0.35$, $P = 4.2$; Mar. 11, $\Delta = 0.39$, $P = 4.7$.

Cionosicys pomiformis (Macf.) Griseb. $\bar{\Delta} = 0.69$, $\bar{P} = 8.4$

Mar. 11, $\Delta = 0.66$, $P = 8.0$; Mar. 11, $\Delta = 0.66$, $P = 8.0$; Mar. 18, $\Delta = 0.76$, $P = 9.1$.

Elaphoglossum latifolium (Sw.) J. Sm. Feb. 11, $\Delta = 0.78$, $P = 9.4$

Epidendrum verrucosum Sw. $\bar{\Delta} = 0.51$, $\bar{P} = 6.1$

Mar. 11, $\Delta = 0.50$, $P = 6.0$; Mar. 18, $\Delta = 0.51$, $P = 6.2$.

Liabum umbellatum (L.) Sch. Bip. $\bar{\Delta} = 0.67$, $\bar{P} = 8.1$

Mar. 11, $\Delta = 0.67$, $P = 8.0$; Mar. 18, $\Delta = 0.67$, $P = 8.1$.

Maurandia erubescens (Zucc.) A. Gray $\bar{\Delta} = 0.80$, $\bar{P} = 9.7$

Feb. 26, $\Delta = 0.75$, $P = 9.0$; Mar. 11, $\Delta = 0.80$, $P = 9.6$; Mar. 18, $\Delta = 0.86$, $P = 10.4$.

Pastinaca sativa L. Mar. 18, $\Delta = 1.16$, $P = 14.0$

Peperomia stellata (Sw.) A. Dietr. $\bar{\Delta} = 0.43$, $\bar{P} = 5.2$

Feb. 11, $\Delta = 0.42$, $P = 5.0$; Feb. 26, $\Delta = 0.40$, $P = 4.8$; Mar. 11, $\Delta = 0.41$, $P = 5.0$; Mar. 18, $\Delta = 0.50$, $P = 6.0$.

Pilea grandifolia (L.) Blume $\bar{\Delta} = 0.63$, $\bar{P} = 7.6$

Feb. 11, $\Delta = 0.60$, $P = 7.3$; Feb. 26, $\Delta = 0.70$, $P = 8.4$; Feb. 26, $\Delta = 0.58$, $P = 7.0$.

Senites Zeugites (L.) Nash Feb. 26, $\Delta = 0.69$, $P = 8.4$

Stenorrhynchus speciosus (Jacq.) L.C. Rich. $\bar{\Delta} = 0.52$, $\bar{P} = 6.3$
Feb. 11, $\Delta = 0.54$, $P = 6.5$; Mar. 11, $\Delta = 0.48$, $P = 5.8$; Mar. 18, $\Delta = 0.54$, $P = 6.5$.

Tradescantia multiflora Sw. Mar. 11, $\Delta = 0.39$, $P = 4.7$

III. *The Ridge Forest*

The ridge forest, closely confined to the main ridge of the mountains and to narrow strips along the crests of the water divides on both windward and leeward slopes, is far more open than that of the slopes or ravines. The vegetation is, therefore, not only more exposed to the influence of light, but is much more wind swept than that of the more deeply and densely covered slopes and ravines. This habitat is, therefore, "relatively xerophilous in the entire make up of its vegetation."

The following are the results:

Ligneous Plants

Acalypha virgata L. $\bar{\Delta} = 0.92$, $\bar{P} = 11.1$
Feb. 9, $\Delta = 0.86$, $P = 10.3$; Mar. 9, $\Delta = 0.96$, $P = 11.6$; Mar. 16, $\Delta = 0.95$, $P = 11.4$.

Acnistus arborescens (L.) Schlecht. Mar. 16, $\Delta = 0.97$, $P = 11.7$

Actinophyllum Sciadophyllum (Sw.) R. C. Schneider Mar. 9, $\Delta = 1.24$, $P = 15.0$

Alchornea latifolia Sw. Mar. 4, $\Delta = 0.89$, $P = 10.8$

Brunfelsia jamaicensis Griseb. $\bar{\Delta} = 0.83$, $\bar{P} = 10.0$
Mar. 9, $\Delta = 0.80$, $P = 9.6$; Mar. 9, $\Delta = 0.90$, $P = 10.8$; Mar. 16, $\Delta = 0.80$, $P = 9.6$.

Cestrum hirtum Sw. Mar. 16, $\Delta = 0.83$, $P = 10.0$

Cinchona sp. $\bar{\Delta} = 1.02$, $\bar{P} = 12.3$
Feb. 20, $\Delta = 1.06$, $P = 12.7$; Mar. 4, $\Delta = 0.96$, $P = 11.6$; Mar. 16, $\Delta = 1.05$, $P = 12.6$.

Citharexylum caudatum L. $\bar{\Delta} = 1.92$, $\bar{P} = 23.1$
Feb. 9, $\Delta = 1.95$, $P = 23.4$; Mar. 9, $\Delta = 2.05$, $P = 24.6$; Mar. 9, $\Delta = 1.77$, $P = 21.3$.

Clethra occidentalis (L.) Steud. $\bar{\Delta} = 0.73$, $\bar{P} = 8.8$
Feb. 9, $\Delta = 0.77$, $P = 9.3$; Mar. 16, $\Delta = 0.68$, $P = 8.2$.

Clusia havetioides (Griseb.) Tr. & Pl. Feb. 18, $\Delta = 0.79$, $P = 9.5$

Cyrilla racemiflora L. Feb. 17, $\Delta = 1.18$, $P = 14.2$
To avoid increasing unduly the number of habitats this determination based on material from John Crow Peak has been included in the Ridge Series.

Dendropanax sp. Feb. 9, $\Delta = 1.00$, $P = 12.0$

Dendropanax nutans (Sw.) Dec. & Pl. $\bar{\Delta} = 0.93$, $\bar{P} = 11.2$
Mar. 9, $\Delta = 0.98$, $P = 11.8$; Mar. 16, $\Delta = 0.87$, $P = 10.5$.

Eugenia virgultosa (Sw.) DC. (?) Feb. 9, $\Delta = 0.72$, $P = 8.7$

Eupatorium glandulosum H.B.K. $\bar{\Delta} = 0.72$, $\bar{P} = 8.7$

Feb. 18, $\Delta = 0.76$, $P = 9.2$; Feb. 20, $\Delta = 0.71$, $P = 8.5$; Mar. 9, $\Delta = 0.69$, $P = 8.4$.

Eupatorium parviflorum Sw. Mar. 9, $\Delta = 0.85$, $P = 10.2$

Eupatorium triste DC. $\bar{\Delta} = 1.24$, $\bar{P} = 14.9$

Mar. 9, $\Delta = 1.26$, $P = 15.1$; Mar. 16, $\Delta = 1.21$, $P = 14.6$.

Gesneria alpina Urban $\bar{\Delta} = 0.58$, $\bar{P} = 7.0$

Feb. 9, $\Delta = 0.60$, $P = 7.2$; Mar. 9, $\Delta = 0.56$, $P = 6.8$.

Guarea Swartzii DC. Mar. 9, $\Delta = 1.07$, $P = 12.8$

Gymnanthes elliptica Sw. Mar. 16, $\Delta = 1.00$, $P = 12.0$

Hedyosmum arborescens Sw. $\bar{\Delta} = 0.73$, $\bar{P} = 8.8$

Mar. 16, $\Delta = 0.73$, $P = 8.8$; Mar. 16, $\Delta = 0.73$, $P = 8.8$.

Mecranium purpurascens (Sw.) Triana $\bar{\Delta} = 0.77$, $\bar{P} = 9.3$

Mar. 4, $\Delta = 0.77$, $P = 9.3$; Mar. 4, $\Delta = 0.77$, $P = 9.2$.

Mettenia globosa (Sw.) Griseb. Mar. 16, $\Delta = 0.87$, $P = 10.5$

Miconia quadrangularis (Sw.) Naud. $\bar{\Delta} = 1.00$, $\bar{P} = 12.1$

Feb. 9, $\Delta = 0.87$, $P = 10.5$; Feb. 20, $\Delta = 0.94$, $P = 11.3$; Mar. 4, $\Delta = 1.05$, $P = 12.7$; Mar. 9, $\Delta = 1.11$, $P = 13.4$; Mar. 16, $\Delta = 0.98$, $P = 11.8$; Mar. 16, $\Delta = 1.07$, $P = 12.9$.

Miconia theaezans (Bonpl.) Cogn. $\bar{\Delta} = 0.88$, $\bar{P} = 10.6$

Feb. 9, $\Delta = 0.84$, $P = 10.1$; Feb. 11, $\Delta = 0.84$, $P = 10.1$; Mar. 11, $\Delta = 0.97$, $P = 11.7$.

Myroxylon nitidum (Hell.) Kuntze $\bar{\Delta} = 1.35$, $\bar{P} = 16.2$

Mar. 9, $\Delta = 1.40$, $P = 16.8$; Mar. 16, $\Delta = 1.14$, $P = 13.7$; Mar. 16, $\Delta = 1.51$, $P = 18.1$.

Ocotea jamaicensis Mez (?) Mar. 4, $\Delta = 1.08$, $P = 13.0$

Palicourea alpina (Sw.) DC. $\bar{\Delta} = 0.69$, $\bar{P} = 8.3$

Feb. 18, $\Delta = 0.55$, $P = 6.6$; Mar. 16, $\Delta = 0.83$, $P = 10.0$.

Pilea Weddellii Fawc. & Rendle Mar. 9, $\Delta = 0.67$, $P = 8.1$

Psychotria corymbosa Sw. $\bar{\Delta} = 0.76$, $\bar{P} = 9.1$

Feb. 9, $\Delta = 0.70$, $P = 8.4$; Mar. 4, $\Delta = 0.75$, $P = 9.1$; Mar. 16, $\Delta = 0.82$, $P = 9.9$.

Psychotria Harrisiana Urban Mar. 16, $\Delta = 0.83$, $P = 10.0$

Rapanea ferruginea (R. & P.) Mez $\bar{\Delta} = 1.02$, $\bar{P} = 12.3$

Feb. 9, $\Delta = 0.96$, $P = 11.6$; Mar. 16, $\Delta = 1.07$, $P = 12.9$.

In the collection of Feb. 9, young leaves gave: $\Delta = 0.89$, $P = 10.7$.

Rhododendron (cultivated) $\bar{\Delta} = 1.04$, $\bar{P} = 12.6$

Feb. 20, $\Delta = 1.01$, $P = 12.2$; Feb. 20, $\Delta = 1.07$, $P = 12.9$.

Solanum punctulatum Dunal. Mar. 13, $\Delta = 1.21$, $P = 14.5$

Vaccinium meridionale Sw. Mar. 16, $\Delta = 1.32$, $P = 15.9$

Young leaves gave: $\Delta = 1.18$, $P = 14.2$.

Wallenia calyptrata Urban $\bar{\Delta} = 0.84$, $\bar{P} = 10.1$

Feb. 9, $\Delta = 0.77$, $P = 9.3$; Mar. 16, $\Delta = 0.91$, $P = 10.9$.

Young leaves were also taken on Feb. 9 and gave: $\Delta = 0.70$, $P = 8.5$.

Herbaceous Plants

Anthurium scandens (Aubl.) Engler $\bar{\Delta} = 0.63$, $\bar{P} = 7.5$
Mar. 9, $\Delta = 0.61$, $P = 7.3$; Mar. 16, $\Delta = 0.64$, $P = 7.6$.
Begonia obliqua L. Mar. 9, $\Delta = 0.33$, $P = 4.0$
Fragaria insularis Rydb. Mar. 9, $\Delta = 1.15$, $P = 13.9$
Liabum umbellatum (L.) Sch. Bip. $\bar{\Delta} = 0.71$, $\bar{P} = 8.5$
Mar. 9, $\Delta = 0.69$, $P = 8.3$; Mar. 16, $\Delta = 0.72$, $P = 8.7$.
Peperomia stellata (Sw.) A. Dietr. Mar. 9, $\Delta = 0.45$, $P = 5.4$
Pilea grandifolia (L.) Blume $\bar{\Delta} = 0.64$, $\bar{P} = 7.7$
Feb. 9, $\Delta = 0.61$, $P = 7.3$; Feb. 18, $\Delta = 0.63$, $P = 7.6$; Mar. 9, $\Delta = 0.67$, $P = 8.1$.
Plantago lanceolata L. Feb. 24, $\Delta = 1.15$, $P = 13.8$
Senites Zeugites (L.) Nash Mar. 9, $\Delta = 0.68$, $P = 8.2$

IV. *Windward Ravines and Slopes*

The windward slopes and ravines, exposed as they are to the direct influence of the moisture-laden trade winds, exhibit in the highest degree the features of climate and vegetation which find their simplest expression in the term *Rain Forest.* The mere statement of the rainfall in inches per year conveys no adequate impression of the actual environment to which the species constituting this vegetation are exposed. The roots of the plants are not merely supplied with water by the heavy and well-distributed rainfall, much of which is stored for long periods in the litter of the forest floor, but the foliage is for much of the time immersed in the floating fog. Thus insolation is much reduced. Even at times when rain is not falling and when the plants are not enveloped in fog, high atmospheric moisture is maintained for long periods of time by evaporation from the litter on the ground and from the moist foliage. Here are large trees with trunks and branches burdened with thin-leaved, succulent-leaved and tank epiphytes, with mats of hepatics and garlands of mosses and filmy ferns, shading a nearly bare forest floor or in other places overtopping a tangled shrubby and herbaceous undergrowth. Any adequate description of this forest would not only outrun the space here available but in view of Shreve's carefully penned description and well chosen and admirably executed plates is quite superfluous. One feature plates cannot depict. This is the reeking wetness of the foliage. This can only be fully appreciated by one who has had the aesthetic pleasure and the physical discomfort of collecting in these forests during or immediately subsequent to the gentle rains, which drip from the glossy foliage, percolate through the sponge-like beds of mosses and hepatics and replenish the tank leaves of the bromeliads, if they are not already overflowing, or in the fog which rolls like clouds of smoke among the trees, covering the leaves like dew.

LIGNEOUS PLANTS

Actinophyllum Sciadophyllum (Sw.) R. C. Schneider $\bar{\Delta} = 1.30$, $\bar{P} = 15.7$

Feb. 4, $\Delta = 1.12$, $P = 13.5$; Mar. 2, $\Delta = 1.48$, $P = 17.8$.

Besleria lutea L. $\bar{\Delta} = 0.58$, $\bar{P} = 7.0$

Feb. 4, $\Delta = 0.48$, $P = 5.7$; Feb. 4, $\Delta = 0.60$, $P = 7.2$; Feb. 13, $\Delta = 0.52$, $P = 6.3$; Feb. 22, $\Delta = 0.59$, $P = 7.1$; Mar. 2, $\Delta = 0.64$, $P = 7.7$; Mar. 13, $\Delta = 0.67$, $P = 8.0$.

Blakea trinervia L. $\bar{\Delta} = 0.58$, $\bar{P} = 6.9$

Feb. 13, $\Delta = 0.42$, $P = 5.0$; Mar. 2, $\Delta = 0.55$, $P = 6.7$; Mar. 2, $\Delta = 0.67$, $P = 8.1$; Mar. 13, $\Delta = 0.66$, $P = 7.9$.

Cestrum hirtum Sw. Mar. 13, $\Delta = 0.72$, $P = 8.7$

Clibadium terebinthinaceum (Sw.) DC. $\bar{\Delta} = 0.60$, $\bar{P} = 7.3$

Feb. 13, $\Delta = 0.48$, $P = 5.8$; Feb. 22, $\Delta = 0.65$, $P = 7.9$; Mar. 3, $\Delta = 0.67$, $P = 8.1$.

Clusia havetioides (Griseb.) Tr. & Pl. $\bar{\Delta} = 0.74$, $\bar{P} = 8.9$

Feb. 20, $\Delta = 0.76$, $P = 9.1$; Feb. 20, $\Delta = 0.72$, $P = 8.6$.

Cyathea furfuracea Baker $\bar{\Delta} = 0.78$, $\bar{P} = 9.5$

Feb. 24, $\Delta = 0.81$, $P = 9.8$; Feb. 24, $\Delta = 0.76$, $P = 9.2$.

Datura suaveolens H. & B. Feb. 13, $\Delta = 0.47$, $P = 5.7$

Dendropanax nutans (Sw.) Dec. & Pl. Mar. 2, $\Delta = 1.06$, $P = 12.8$

Young leaves from the same tree gave slightly lower values: $\Delta = 0.91$, $P = 10.9$.

Eupatorium glandulosum H. B. K. $\bar{\Delta} = 0.64$, $\bar{P} = 7.7$

Feb. 22, $\Delta = 0.54$, $P = 6.5$; Mar. 2, $\Delta = 0.62$, $P = 7.4$; Mar. 4, $\Delta = 0.76$, $P = 9.1$.

Eupatorium parviflorum Sw. Mar. 2, $\Delta = 1.07$, $P = 129.$

Eupatorium riparium Regel $\bar{\Delta} = 0.58$, $\bar{P} = 7.1$

Mar. 2, $\Delta = 0.55$, $P = 6.7$; Mar. 13, $\Delta = 0.61$, $P = 7.4$.

Gesneria alpina Urban $\bar{\Delta} = 0.51$, $\bar{P} = 6.2$

Mar. 2, $\Delta = 0.50$, $P = 6.1$; Mar. 13, $\Delta = 0.52$, $P = 6.3$.

Guarea Swartzii DC. $\bar{\Delta} = 0.83$, $\bar{P} = 10.0$

Feb. 13, $\Delta = 0.73$, $P = 8.8$; Feb. 22, $\Delta = 0.91$, $P = 11.0$; Mar. 13, $\Delta = 0.84$, $P = 10.1$.

Hedyosmum arborescens Sw. $\bar{\Delta} = 0.65$, $\bar{P} = 7.9$

Feb. 4, $\Delta = 0.56$, $P = 6.8$; Feb. 4, $\Delta = 0.49$, $P = 5.9$; Feb. 13, $\Delta = 0.55$, $P = 6.7$; Feb. 20, $\Delta = 0.64$, $P = 7.7$; Mar. 2, $\Delta = 0.66$, $P = 8.0$; Mar. 2, $\Delta = 0.70$, $P = 8.4$; Mar. 4, $\Delta = 0.65$, $P = 7.8$; Mar. 13, $\Delta = 0.82$, $P = 9.9$; Mar. 13, $\Delta = 0.82$, $P = 9.9$.

Marcgravia Brownei (Tr. & Pl.) Krug. & Urban. $\bar{\Delta} = 0.78$, $\bar{P} = 9.4$

Feb. 4, $\Delta = 0.62$, $P = 7.5$; Feb. 13, $\Delta = 0.65$, $P = 7.8$; Feb. 22, $\Delta = 0.67$, $P = 8.1$; Mar. 2, $\Delta = 0.85$, $P = 10.2$; Mar. 4, $\Delta = 0.87$, $P = 10.5$; Mar. 13, $\Delta = 1.02$, $P = 12.2$.

The foregoing determinations on which the average for the species is based are from the leaves of the aerial branches, extending from the trunks. Two determinations on the "juvenile" leaves of the creeping stems were secured. These are:

Feb. 13, $\Delta = 0.53$, $P = 6.4$; Mar. 4, $\Delta = 0.71$, $P = 8.5$.

Meriania purpurea Sw. $\overline{\Delta} = 0.87$, $\overline{P} = 10.5$

Feb. 22, $\Delta = 0.77$, $P = 9.3$; Mar. 2, $\Delta = 0.90$, $P = 10.8$; Mar. 13, $\Delta = 0.95$, $P = 11.5$.

Miconia quadrangularis (Sw.) Naud. Mar. 13, $\Delta = 0.97$, $P = 11.7$

Miconia theaezans (Bonpl.) Cogn. $\overline{\Delta} = 0.90$, $\overline{P} = 10.8$

Feb. 4, $\Delta = 0.76$, $P = 9.1$; Mar. 13, $\Delta = 0.97$, $P = 11.7$; Mar. 13, $\Delta = 0.96$, $P = 11.5$.

Palicourea alpina (Sw.) DC. $\overline{\Delta} = 0.69$, $\overline{P} = 8.3$

Feb. 13, $\Delta = 0.52$, $P = 6.3$; Feb. 24, $\Delta = 0.78$, $P = 9.3$; Mar. 2, $\Delta = 0.63$, $P = 7.6$; Mar. 4, $\Delta = 0.75$, $P = 9.0$; Mar. 13, $\Delta = 0.77$, $P = 9.2$.

Pilea Weddellii Fawc. & Rendle $\overline{\Delta} = 0.62$, $\overline{P} = 7.4$

Feb. 22, $\Delta = 0.57$, $P = 6.8$; Feb. 24, $\Delta = 0.67$, $P = 8.1$; Mar. 4, $\Delta = 0.61$, $P = 7.3$.

Piper hispidum Sw. $\overline{\Delta} = 0.50$, $\overline{P} = 6.1$

Feb. 13, $\Delta = 0.43$, $P = 5.2$; Feb. 22, $\Delta = 0.45$, $P = 5.4$; Mar. 13, $\Delta = 0.63$, $P = 7.6$.

Podocarpus Urbani Pilger Feb. 24, $\Delta = 0.93$, $P = 11.2$

Young leaves gave: $\Delta = 0.81$, $P = 9.7$.

Psychotria corymbosa Sw. $\overline{\Delta} = 0.76$, $\overline{P} = 9.2$

Feb. 20, $\Delta = 0.75$, $P = 9.0$; Mar. 4, $\Delta = 0.67$, $P = 8.1$; Mar. 13, $\Delta = 0.86$, $P = 10.4$.

Schradera involucrata (Sw.) Schum. Mar. 13, $\Delta = 1.24$, $P = 15.0$

Solanum punctulatum Dunal Mar. 13, $\Delta = 1.14$, $P = 13.8$

Tovaria pendula R. & P. $\overline{\Delta} = 0.70$, $\overline{P} = 8.5$

Feb. 13, $\Delta = 0.68$, $P = 8.2$; Feb. 22, $\Delta = 0.72$, $P = 8.7$.

Vaccinium meridionale Sw. $\overline{\Delta} = 1.33$, $\overline{P} = 16.1$

Mar. 2, $\Delta = 1.31$, $P = 15.8$; Mar. 13, $\Delta = 1.36$, $P = 16.3$.

HERBACEOUS PLANTS

Anthurium scandens (Aubl.) Engler $\overline{\Delta} = 0.52$, $\overline{P} = 6.3$

Feb. 13, $\Delta = 0.50$, $P = 6.0$; Mar. 4, $\Delta = 0.52$, $P = 6.2$; Mar. 13, $\Delta = 0.55$, $P = 6.6$.

Begonia glabra Aubl. $\overline{\Delta} = 0.30$, $\overline{P} = 3.5$

Feb. 4, $\Delta = 0.29$, $P = 3.4$; Feb. 13, $\Delta = 0.30$, $P = 3.6$.

Begonia obliqua L. $\overline{\Delta} = 0.33$, $\overline{P} = 3.9$

Feb. 20, $\Delta = 0.31$, $P = 3.7$; Feb. 24, $\Delta = 0.33$, $P = 4.0$; Mar. 2, $\Delta = 0.35$, $P = 4.2$; Mar. 4, $\Delta = 0.32$, $P = 3.8$; Mar. 13, $\Delta = 0.35$, $P = 4.2$; Mar. 13, $\Delta = 0.31$, $P = 3.7$.

Elaphoglossum chartaceum Baker Mar. 13, $\Delta = 0.96$, $P = 11.5$

Fragaria insularis Rydb. Mar. 13, $\Delta = 1.09$, $P = 13.1$

Gesneria mimuloides (Griseb.) Urban $\overline{\Delta} = 0.44$, $\overline{P} = 5.2$

Mar. 2, $\Delta = 0.42$, $P = 5.0$; Mar. 4, $\Delta = 0.45$, $P = 5.4$.

Liabum umbellatum (L.) Sch. Bip. $\overline{\Delta} = 0.58, \overline{P} = 7.0$
Mar. 2, $\Delta = 0.53, P = 6.4$; Mar. 4, $\Delta = 0.60, P = 7.2$; Mar. 13, $\Delta = 0.62, P = 7.5$.

Lobelia assurgens L. $\overline{\Delta} = 0.73, \overline{P} = 8.7$
Feb. 13, $\Delta = 0.66, P = 8.0$; Mar. 2, $\Delta = 0.76, P = 9.1$; Mar. 2, $\Delta = 0.76, P = 9.1$.

Panicum palmifolium Poir. $\overline{\Delta} = 0.80, \overline{P} = 9.6$
Feb. 4, $\Delta = 0.76, P = 9.2$; Feb. 22, $\Delta = 0.80, P = 9.7$; Mar. 2, $\Delta = 0.83, P = 10.0$.

Peperomia stellata (Sw.) A. Dietr. $\overline{\Delta} = 0.42, \overline{P} = 5.1$
Mar. 2, $\Delta = 0.42, P = 5.1$; Mar. 4, $\Delta = 0.41, P = 4.9$; Mar. 13, $\Delta = 0.43, P = 5.2$.

Pilea Mar. 4, $\Delta = 0.65, P = 7.9$

Pilea grandifolia (L.) Blume $\overline{\Delta} = 0.58, \overline{P} = 7.0$
Feb. 13, $\Delta = 0.57, P = 6.8$; Feb. 22, $\Delta = 0.59, P = 7.1$.

Pilea nigrescens Urban $\overline{\Delta} = 0.57, \overline{P} = 6.9$
Feb. 20, $\Delta = 0.56, P = 6.7$; Feb. 22, $\Delta = 0.51, P = 6.1$; Feb. 24, $\Delta = 0.55, P = 6.6$; Mar. 2, $\Delta = 0.58, P = 6.9$; Mar. 4, $\Delta = 0.61, P = 7.4$; Mar. 13, $\Delta = 0.61, P = 7.4$.

Prescottia stachyodes (Sw.) Lindl. $\overline{\Delta} = 0.81, \overline{P} = 9.7$
Feb. 24, $\Delta = 0.84, P = 10.1$; Mar. 4, $\Delta = 0.72, P = 8.6$; Mar. 13, $\Delta = 0.87, P = 10.5$.

Senites Zeugites (L.) Nash $\overline{\Delta} = 0.62, \overline{P} = 7.4$
Feb. 24, $\Delta = 0.59, P = 7.1$; Mar. 4, $\Delta = 0.64, P = 7.8$.

III. Discussion of Results

In analyzing these data we shall consider three main problems:

A. The relationship between growth form and osmotic concentration.

B. The differentiation of the habitats of the Blue Mountains in osmotic concentration.

C. The relative value of the osmotic concentration of the fluids of the plants of the Blue Mountain rain forest as compared with other phytogeographically different areas which have been investigated by similar methods.

The only method by which these problems may be investigated is the statistical one, the comparison by means of averages of different sections of the data.

The averages of species means (or of species determinations, when only one for a habitat is available) are given for herbaceous and ligneous plants separately, and for all plants, for each of the four habitats in Table I.

TABLE I

Fundamental Averages for Blue Mountain Rain Forest

Habitats and Constants	Ligneous Plants		Herbaceous Plants		Ligneous and Herbaceous Plants	
	Number	Mean	Number	Mean	Number	Mean
I. Ruinate of leeward slopes:						
Freezing-point lowering......	40	1.089	17	.812	57	1.007
Osmotic concentration.......		13.05		9.77		12.07
II. Leeward ravines:						
Freezing-point lowering......	32	.901	13	.628	45	.822
Osmotic concentration.......		10.83		7.59		9.89
III. The ridge forest:						
Freezing-point lowering......	36	.958	8	.718	44	.914
Osmotic concentration.......		11.54		8.63		11.01
IV. Windward ravines and slopes:						
Freezing-point lowering......	28	.805	15	.627	43	.743
Osmotic concentration.......		9.73		7.52		8.96
I–IV. All species:						
Freezing-point lowering......	136	.952	53	.700	189	.881
Osmotic concentration.......		11.44		8.80		10.59

These are the fundamental constants upon which much of the following discussion must be based.

Comparison of Ligneous and Herbaceous Growth Forms.—The justification for the division of the determinations into those for herbaceous and those for ligneous plants is clearly brought out by Table I. For each habitat studied the freezing point lowering is on the average lower for the herbaceous than for the ligneous plants. The actual differences in terms of atmospheres are given in Table II.

TABLE II

Comparison of Osmotic Concentration of Herbaceous and Ligneous Growth Forms

Growth Form	Ruinate of the Leeward Slopes	Leeward Ravines	Ridge Forest	Windward Slopes and Ravines	All Habitats
All species....................	12.07	9.89	11.01	8.96	10.59
Ligneous species..............	13.05	10.83	11.54	9.73	11.44
Herbaceous species............	9.77	7.59	8.63	7.52	8.80
Difference....................	3.28	3.24	2.91	2.21	2.64
Percentage difference..........	25.13	29.92	25.22	22.71	23.08

Thus the difference in the concentration of the sap of ligneous and herbaceous plants is from about 23 to about 30 percent of the higher value, that for ligneous forms.

Comparison of Habitats in the Blue Mountain Region.—Turning now to the comparisons of the local habitats among themselves we note the following points which must be taken into consideration in the analysis of the data.

The comparisons between the windward and the leeward exposures on the basis of the now available data may be expected to give a minimum rather than a maximum measure of the differences between them. This is true for three reasons. First, we have made the comparison between the plants of the windward slopes and windward ravines taken together and two of the sub-habitats of the leeward slopes. Thus if there be measurable differences between the sap properties of the windward ravines and the windward slopes, the combination of the two will tend to minimize the differences which might have been obtained had it been practicable to deal separately with the properties of the saps of the windward slopes and ravines. Second, we have arbitrarily excluded a great number of forms which are apparently the most hygrophilous and are possibly characterized by an even lower osmotic concentration than are the species for which determinations are given in these pages. Had it been possible to free the mats or festoons of certain of the cryptogamic epiphytes from the superficial water with which they are so constantly saturated, without modifying the concentration of their tissue solutions by drying, we believe that a series of determinations falling almost if not entirely in the lower range of variation in osmotic concentration as shown by the available determinations might have been obtained. Third, to render the results from the Blue Mountain habitats as nearly as possible comparable with others which have been or are being investigated we have excluded the Bromeliaceae, the Orchidaceae, with the exception of truly terrestrial forms, and some other phanerogamic epiphytes. There is, as far as we are aware, no *a priori* reason to consider that these forms would be characterized by low osmotic concentrations. While the detailed discussion of these ecologically most interesting forms is reserved for a comparative study to be published later, it may be said in passing that the concentration of these forms has been found to be usually far lower than that of other species of the vegetation.

These facts while they must detract somewhat from our constants as an exact description of the region in question, make differences secured under these limitations much more significant.

In considering differences in sap concentration in relation to local habitats in the rain-forest region the comparison of each of the four habitats with the three others may be made in detail in a series of four tables.

In view of the differentiation between herbaceous and ligneous plants demonstrated above, the comparison must first be made for each class separately.

In these tables each of which is devoted to the values showing the absolute and relative magnitudes of the constants of a given habitat, the comparisons are made in two ways. First, the actual differences in mean osmotic concentration, $\overline{P}$, between any habitat and the three other habitats have been determined. These are the values with signs. Second, the ratio of the mean osmotic concentration of the sap of every habitat to that of each other habitat with which it is to be compared has been determined. These are the values given in black-faced type.

The first method has the obvious advantage that differences are expressed in the concrete terms of osmotic concentration. Relative values, as employed in the second method, are on the other hand more convenient for comparison. The exact method of drawing the comparisons will be clear from an explanation of the individual tables.

The first column of Table III, in which the values obtained in the ruinate of the leeward slopes are compared with those of each of the other habitats, gives the growth forms on which the comparisons are based. It has been practicable to recognize only two of these in the Blue Mountain region, the herbs and arborescent, frutescent and suffrutescent plants. The second column gives the actual mean values in atmospheres of the plants of the ruinate. The third to

Table III

Growth Form	Ruinate of the Leeward Slopes	Ruinate of the Leeward Slopes Compared with Other Habitats			
		Leeward Ravines	Ridge Forest	Windward Slopes and Ravines	All Habitats
Herbaceous. . . .	9.77	+2.18	+1.14	+2.25	+0.97
	1.00	**1.29**	**1.13**	**1.30**	**1.11**
Ligneous.	13.05	+2.22	+1.51	+3.32	+1.61
	1.00	**1.20**	**1.13**	**1.34**	**1.14**
All species.	12.07	+2.18	+1.06	+3.11	+1.48
	1.00	**1.22**	**1.10**	**1.35**	**1.14**

sixth columns contain the actual differences between the mean values for the plants of the ruinate and of the three other habitats and of the region as a whole. These are obtained by subtracting the values for each of the habitats compared from the values for the ruinate as given in the second column. The same method is followed in drawing up the three other comparison tables to be discussed below.

The signs of the differences are positive throughout. Thus the concentrations prevailing in the plants of the ruinate, which has been recognized by Shreve and others as the most xerophilous of the Blue Mountain habitats, are higher for both herbaceous and ligneous plants and for all species of plants than those in any other habitat. They are over two atmospheres higher than those found in the plants of the neighboring leeward ravines, over one atmosphere higher than those of the ridge forest and from over two to more than three atmospheres higher than those demonstrated on the windward side of the range.

The relative values, obtained by dividing the mean concentration of the plants of the ruinate by those of each of the other habitats, show that the concentration of the sap of the plants of the most xerophytic of the habitats is from about 20 to 30 percent more concentrated than that of the leeward ravines, about 10–13 percent more concentrated than that of the ridge forest, and from 30 to 35 percent more concentrated than that of the plants of the windward habitats.

Table IV, giving the relationship between the sap properties of the plants of the ridge forest and those of the other habitats, shows that the plants of this habitat have a concentration lower than that of the comparable growth forms of the ruinate but higher than that of either the leeward ravines or the windward ravines and slopes. The amount of the difference is as great as 2 atmospheres in one case only.

TABLE IV

Growth Form	Ridge Forest	Ridge Forest Compared with Other Habitats			
		Ruinate of Leeward Slopes	Leeward Ravines	Windward Slopes and Ravines	All Habitats
Herbaceous....	8.63	−1.14	+1.04	+1.11	−0.17
	1.00	0.88	1.14	1.15	0.98
Ligneous.......	11.54	−1.51	+0.71	+1.81	+0.10
	1.00	0.88	1.15	1.19	1.01
All species.....	11.01	−1.06	+1.12	+2.05	+0.42
	1.00	0.91	1.11	1.23	1.04

The relative differences are not large. In only a single comparison does the ratio indicate a difference of as much as 23 percent.

Table V shows that the concentration in the plants of the leeward ravines is lower than in those of the ruinate or of the ridge forest but higher than that of the windward slopes and ravines. The differences between the concentrations in the leeward ravines and on the ridges on the one hand and between the leeward ravines and the windward ravines on the other are not large.

TABLE V

Growth Form	Leeward Ravines	Leeward Ravines Compared with Other Habitats			
		Ruinate of Leeward Slopes	Ridge Forest	Windward Slopes and Ravines	All Habitats
Herbaceous....	7.59	−2.18	−1.04	+0.07	−1.21
	1.00	**0.78**	**0.88**	**1.01**	**0.86**
Ligneous.......	10.83	−2.22	−0.71	+1.10	−0.61
	1.00	**0.83**	**0.94**	**1.11**	**0.95**
All species.....	9.89	−2.18	−1.12	+0.93	−0.70
	1.00	**0.82**	**0.90**	**1.10**	**0.93**

The final comparison is that of the windward ravines and slopes with the other habitats. This is made in Table VI. The differences show that the plants of the most hygrophytic habitat of the region under investigation are characterized by a lower osmotic concentration than those of any other habitat. To this rule there is not a single exception. The values range from 74 to 99 percent of that of other habitats.

TABLE VI

Growth Form	Windward Slopes and Ravines	Windward Slopes and Ravines Compared with Other Habitats			
		Ruinate of Leeward Slopes	Leeward Ravines	Ridge Forest	All Habitats
Herbaceous....	7.52	−2.25	−0.07	−1.11	−1.28
	1.00	**0.77**	**0.99**	**0.87**	**0.85**
Ligneous.......	9.73	−3.32	−1.10	−1.81	−1.71
	1.00	**0.75**	**0.90**	**0.84**	**0.85**
All species.....	8.96	−3.11	−0.93	−2.05	−1.63
	1.00	**0.74**	**0.91**	**0.81**	**0.85**

Comparison of Blue Mountain Rain Forest with Other Regions.—Our work in Jamaica was undertaken primarily to secure determinations from an extremely hygrophytic habitat for comparison with the xerophytic region about the Desert Laboratory at Tucson and the more mesophytic vegetation in the neighborhood of the Station for Experimental Evolution on Long Island.

Since carrying out the Jamaican determinations we have been able to make very substantial beginnings on the investigation of several other habitats, for example the forests of the upper Santa Catalina mountains and the various transition stations to the desert floor in southern Arizona, the Everglades, the Pinelands, and the hammocks of sub-tropical Florida, rich in West Indian species. A detailed comparison of the montane rain forest with other regions may profitably be reserved until the completion of these studies. In the meantime it is worth while to indicate to phytogeographers and ecologists the relative position of the Blue Mountain habitats in the series concerning which published data are available.

Consider first the values for the rain-forest plants as compared with those obtained in more mesophytic regions. Two such series are available, that of Ohlweiler ('12) based on trees and shrubs growing at the Missouri Botanical Garden, and that of Harris, Lawrence and Gortner ('15) for Long Island habitats.

Ohlweiler's St. Louis series suffers from two disadvantages as regarded from the standpoint of this paper. First, it is based upon a series of species brought together from various natural habitats and cultivated in a botanical garden. All the species were, however, capable of growth in the open under the conditions prevailing at St. Louis. Second, sap was extracted without antecedent freezing of the leaf tissue. As a result the freezing-point lowerings recorded are probably too low.

Ohlweiler's series comprises trees and shrubs only. Comparing with the general average for ligneous plants from the Blue Mountains the results are:

	Means
St. Louis series	14.96
Blue Mountain series	11.44

The trees and shrubs growing in the Botanical Garden at St. Louis show, therefore, a concentration of their leaf sap of from 2 to 5 atmospheres higher than do those of the various Blue Mountain habitats, and over 3 atmospheres more than the average for the Blue Mountain region as a whole.

The averages for the Long Island series[2] have been calculated for individual habitats. The averages for both trees and shrubs and for herbaceous plants may be compared with the individual Blue Mountain habitats. The means of the accompanying tables, VII–VIII,

TABLE VII

Comparison of Ligneous Plants

Jamaican Habitats		Long Island Habitats	
Ruinate	13.05	13.34	Beaches, coastal sand dunes and marshes
Ridge forest	11.54	14.64	Dryer woods and open fields
Leeward ravines	10.83		
Windward habitats	9.73	14.07	Permanently moist localities
All habitats	11.44	14.40	All habitats

TABLE VIII

Comparison of Herbaceous Plants

Jamaican Habitats		Long Island Habitats	
Ruinate	9.77	13.62	Beaches, coastal sand dunes and marshes
Ridge forest	8.63	10.04	Dryer woods and open fields
Leeward ravines	7.59		
Windward habitats	7.52	9.27	Permanently moist localities
All habitats	8.80	10.41	All habitats

show that with the exception of the herbaceous plants of the ruinate there is no habitat of the Blue Mountain region which exhibits an osmotic concentration of the leaf sap of the species constituting its flora as high as the lowest mean found in the Cold Spring Harbor series. The herbaceous plants of the ruinate—the most xerophytic of the Blue Mountain habitats—show a concentration slightly higher than those of the Long Island habitats which are constantly moist, *i. e.*, fresh water bogs, lake shores and springy hillsides.

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[2] The values given for Cold Spring Harbor are preliminary averages of determinations, not of species means, made in 1914 by Harris, Lawrence and Gortner. They will be replaced later by averages based on far larger series of determinations made in 1915 by Lawrence and Harris, and on subsequent determinations by Harris.

In view of the fact that the Long Island series here used is to be much increased, further discussion of the observed differences may be postponed until the more extensive data are worked up.

A conspicuous difference in the osmotic concentration of rain-forest and desert vegetation is of course to be expected after the demonstration of the differentiation of the sap properties of the plants of this and more mesophytic regions. Two fairly satisfactory sets of determinations for deserts are now available. The magnitude of the differences between the rain forest and these will give some indication of the range of variation to be found in the mean osmotic concentration of the fluids of the species of different vegetations.

A comparison with the Arizona desert series of determinations made at the time of hibernal and vernal vegetative activity[3] is made in the accompanying tables, IX–X. In these, averages[4] are given for

TABLE IX

Comparison for Ligneous Perennials

Jamaican Habitats		Arizona Habitats	
Ruinate	13.05	22.01	Rocky slopes
Ridge forest	11.54	21.04	Canyons
Leeward ravines	10.83	17.30	Arroyos
Windward habitats	9.73		
		30.34	Bajada slopes
		45.20	Salt spots
All habitats	11.44	24.97	All habitats

TABLE X

Comparison for Herbaceous Plants

Jamaican Habitats		Arizona Habitats	
Ruinate	9.77	15.94	Rocky slopes
Ridge forest	8.63	13.33	Canyons
Leeward ravines	7.59	12.99	Arroyos
Windward habitats	7.52		
		20.53	Bajada slopes
		23.57	Salt spots
All habitats	8.80	15.15	All habitats

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[3] Studies on the summer vegetation have been made and will eventually be published.

[4] The averages for the southwestern deserts are based on species determinations, not on means of determinations as in the Jamaica series. The difference in method is of no significance for present purposes.

each of the sub-habitats for both ligneous and herbaceous forms. In our original paper (Harris, Lawrence and Gortner, 1916) the determinations for the ligneous plants are further subdivided into trees and shrubs as one class and dwarf shrubs, half shrubs and woody twiners as the other. Such distinctions have not been so easily made in the rain forest. The two groups of desert ligneous perennials have, therefore, been combined to render them more comparable with the Jamaica ligneous perennials.

The Arizona herbaceous plants were originally divided into the two very distinct groups, winter annuals and perennial herbs. These have also been combined to render them more nearly comparable with the herbaceou plants of the Blue Mountain region.

Of course no one of the desert habitats is at all similar to those of the Blue Mountains. Those which are least of all comparable, the bajadas and the salt spots, have been set off from the others.

The tables show at a glance that the concentrations of the desert are from fifty to nearly two hundred percent higher for individual habitats in the Arizona deserts than in the Jamaica Blue Mountains.

The differences between the two regions are strikingly exemplified by a comparison of the herbaceous plants of the desert with the ligneous plants of the rain forest. The *minimum* osmotic concentration in desert herbaceous plants (12.99 atmospheres in the arroyos) is practically as high as the *maximum* concentration for ligneous plants in the Blue Mountains (13.05 atmospheres in the ruinate). The mean concentration for herbaceous plants in the desert is 15.15 atmospheres as compared with 11.44 atmospheres, the mean concentration of ligneous plants in the Blue Mountains.

While logically a comparison of the rain-forest vegetation of the Blue Mountains with the desert vegetation of the coastal deserts has no greater significance than that with the vegetation of the Arizona deserts it will, because of the relatively short distance separating the two Jamaican habitats, have a greater interest for most readers.

The comparison with the coastal desert of the southern shore of Jamaica (Harris and Lawrence, 1917) must be limited to ligneous perennials. The average of the 31 species means for arborescent and suffrutescent plants of the coastal desert, omitting only the herbaceous *Sesuvium*, *Bromelia*, *Bryophyllum* and the Cacti, is 30.05 atmospheres, as compared with 11.44 atmospheres for the montane habitats!

Very high concentrations are also found in the mangrove swamps on the southern shore of Jamaica (Harris and Lawrence, 1917*b*). Thus *Rhizophora Mangle* shows concentrations ranging from 29.2 to 30.9 atmospheres, *Laguncularia racemosa* shows concentrations ranging from 24.6 to 34.8 atmospheres and *Avicennia nitida* yields values from 41.5 to 54.4 atmospheres.

IV. Recapitulation

The Blue Mountains of Jamaica, intercepting as they do the trade winds in their sweep across the Caribbean Sea, exhibit a conspicuous differentiation in the flora and especially in the vegetation of the windward (northern) and the leeward (southern) sides of the range.

The windward slopes, and especially the windward ravines, exhibit all those features of vegetation and of structure of the constituent species which are called to the mind of the botanist by the term *Rain Forest*. In the higher mountains the leeward ravines share many of the characteristics of the windward ravines and slopes, but the leeward slopes, and especially the scrub formation known as ruinate, are far more xerophytic in their botanical characteristics.

The subalpine ridges, while lacking some of the most characteristic and typical xerophytic species of the ruinate, are nevertheless clearly far more xerophytic than either the windward slopes or ravines or the leeward ravines.

These differences have long been known to botanists, and have recently been splendidly described and illustrated by Shreve.

The purpose of the investigations described in this paper, which is one of a series on the sap properties of the plant species of diverse vegetations, is to present the results of an extensive series of cryoscopic determinations of osmotic concentration of leaf sap in the species of the Blue Mountains, to compare these habitats among themselves on the basis of the average osmotic concentration of their leaf tissue fluids, and to compare the region as a whole with other areas, mesophytic and xerophytic, which have been investigated in a similar manner.

The results of the present study confirm the conclusions concerning the existence of a higher osmotic concentration in the tissue fluids of the leaves of ligneous than in those of the tissue fluids of herbaceous plants, drawn from the investigation of the deserts of southern Arizona. The difference between the concentration of the sap of the two groups of growth forms is clearly marked in the series of determinations from each of the Blue Mountain habitats. The differences are not, however, so large as those demonstrated in the desert series.

The four sub-habitats, recognized in the Blue Mountains, show distinct differences in the osmotic concentration of their tissue fluids.

The ruinate, which has been regarded by ecologists as the most xerophytic of the habitats, shows a distinctly higher osmotic concentration of the leaf tissue fluids of its constituent species than any other habitat. The plants of the ridge forest show a higher osmotic concentration than do those of the leeward ravines and the windward ravines and slopes, but lower than that of the plants of the ruinate. The leeward ravines are characterized by plants with lower osmotic concentration than the vegetation of the ruinate and of the ridge forest, but higher than that of the windward ravines and slopes. Finally, the windward habitats, which are the most hygrophilous of the region, are characterized by a sap concentration lower than that of any other habitat.

The osmotic concentration in the sap of the plants of the Blue Mountains is the lowest of that of any region as yet extensively investigated. The ligneous forms show an average concentration of about 11.44 atmospheres as compared with 14.96 atmospheres in Ohlweiler's St. Louis series and 14.40 for our own preliminary series from Long Island habitats. The average concentration for herbaceous plants in the Blue Mountains is about 8.80 atmospheres as compared with 10.41 atmospheres from our preliminary Long Island series.

Comparisons with desert regions show much more striking differences. Thus the herbaceous plants of the rain forest show an average concentration of 8.80 atmospheres as compared with 15.15 atmospheres in the herbaceous plants of the winter flora of the deserts around Tucson. The ligneous plants of the rain forest have a concentration of only about 11.44 atmospheres as contrasted with 24.97 atmospheres in the series of ligneous plants investigated in our southwestern deserts. The Jamaican coastal deserts show slightly higher concentration even than those of the Arizona series.

While these general averages are the simplest expression of the differences between these regions, they are by no means an adequate description. They conceal the differences which obtain in each of the areas investigated. For a more adequate conception of the conditions, the reader must turn to the more detailed comparisons which are made possible by the data presented earlier in these pages, and in the original papers to which reference has been made.

Further comparisons will be made when the data from other field work are properly arranged for discussion.

CARNEGIE INSTITUTION OF WASHINGTON

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THE VIABILITY OF RADISH SEEDS (RAPHANUS SATIVUS L.) AS AFFECTED BY HIGH TEMPERATURES AND WATER CONTENT

H. D. Waggoner

The first important work on the effect of high temperatures upon the viability of seeds was done by Edwards and Colin in 1834. From that date up to the present time considerable interest has been shown along this line and a large and valuable literature has accumulated. A large majority of the earlier workers in this field were interested in the maximum temperatures that seeds are able to withstand, and paid but little attention to the cause of the loss of vitality when seeds were heated at temperatures above their maximum. Recently several investigators have studied the effect of high temperatures upon protoplasm, and important contributions have appeared that throw much light upon the real cause of death in seeds.

It seemed advisable, owing to the lack of time, to confine the present paper to a detailed study of the effect of high temperatures upon the germinating power of seeds and to reserve the consideration of the direct effect of heat upon the living protoplasm of seeds for a subsequent study.

A close study of the results obtained by earlier workers shows a wide difference in the maximum temperatures that the same or nearly related seeds can endure without injury. It further reveals the interesting fact that an intimate relation exists between the method employed in treating the seeds and the temperature at which serious injury or death occurs. Briefly, the methods heretofore employed are as follows: (1) The seeds were heated in water or in an atmosphere saturated with moisture. By this mode of treatment it is apparent that the seeds absorbed more or less water during the heating process. Authors using this method invariably report a low lethal temperature for the seeds used. (2) The seeds were heated in small closed containers. By this method the seeds during the process of heating gave up moisture to a greater or less extent until an equilibrium between the imbibition energy of the seeds and the vapor tension of the inclosed air was established. The results obtained by this method varied according to the amount of water present in the seeds when heated. (3) The seeds were heated in ovens. In this case it is evident that the air in the ovens was of low relative humidity and consequently the seeds lost moisture during the heating. Seed treated in this manner endure exceedingly high temperatures without apparent injury.

It seemed desirable to make a detailed study of the resistance of seeds of different water contents to high temperatures under carefully controlled conditions. Accordingly, experiments were carried on in the laboratories of plant physiology under the direction of Professor Chas. F. Hottes, to whom the writer is greatly indebted for searching criticism and helpful suggestion.

In taking up this subject anew two closely related lines of experimentation were outlined. The one was concerned with the effect of high temperature upon series of samples of seeds of increasing water content. The other was to determine the cause for the wide difference in the resistance of seeds treated by the three methods indicated above. A survey of the more pertinent literature will serve to bring these two points definitely before us.

As early as 1859, Heiden reported that grains of barley, when exposed for one hour to dry air at 90° C. germinated, while similar grains heated in water at 60° C. for the same period of time were killed. In 1865, Fiedler (Sachs), working with seeds of pea, rye, flax, barley and corn, showed that swollen seeds were killed at from 50° C. to 60° C., while those containing less moisture withstood 70° C. or more. The seeds were treated in closed test tubes immersed in water maintained at the desired temperature. Von Höhnel (1877) improved the method as used by Fiedler in that he covered the seeds in the test tubes with fine metal filings to facilitate the transfer of heat. He worked with seeds of a number of different species and reported that most of them when sufficiently dried were able to endure an exposure at 110° C. for sixty minutes. Some heated at 125° C. for fifteen minutes were found viable. Just (1875, 1877) reports that clover seeds heated in a saturated atmosphere at 50° C. for forty-eight hours, or at 75° C. for one hour, lost their viability. However, if the seeds were well dried they could endure a temperature of 120° C. Other kinds of seeds gave similar results. Detmer (1880) records that the viability of seeds is not lost in boiling water, provided they are not in a swollen state. He further states that the less moisture seeds contain at the time of treatment the greater is their resistance to high temperatures. Other investigators of this early period obtained similar results.

More recently, Jodin (1899) found that seeds of peas and cress when dried at 60° C. for twenty-four hours can be heated at 98° C. in the dry air of sealed tubes for six hours and still retain their viability; similar seeds heated in humid air at 40° C. lost their viability in twenty hours. In subsequent experiments he found that the resistance of the seeds of pea and cress was increased in a marked degree when calcium chloride was introduced into the tube with the seeds. He states that seeds may be exposed to dry air at 65° C. for prolonged periods without loss of vitality, but adds that this may be done only if one heats them in an open dish to permit a rapid loss of water from the seeds. If the air becomes saturated with water vapor, the seeds can endure only a comparatively low temperature without injury. Dixon (1902) dried various kinds of seeds over sulphuric acid and later in an oven at 95° C. for several days without destroying their viability. He treated the samples in closed test tubes and found that when sufficiently dry the seeds could withstand temperatures far above that of boiling water (110° C.–120° C.) without injury. Neuberger (1914) and others of recent date, in their studies on the comparative resistance of moist and dry seeds to high temperatures, in a general way, confirm the results of the earlier authors.

Pouchet (1866), working with seeds of Medicago obtained from sheep-wool brought from South America, found that they germinated after being exposed to boiling water for four hours. He subsequently experimented with other Medicago seeds and found that only those germinated which, after a prolonged treatment in boiling water, did not swell. Nobbe (1876) confirmed Pouchet's results. Dixon (1901) and Schneider-Orelli (1909, 1910) attributed the high resistance of these seeds to the fact that many of the seed coats are impermeable to water.

From the above review, it is apparent that considerable work has been done on the resistance of seeds to high temperatures. The experiments have been carried on with seeds containing widely different and undetermined amounts of water. As far as the writer has been able to determine no one has attempted to study, in series, the resistance of the same kind of seeds containing definite and known quantities of water at the time of heating. It is only through a quantitative study of this kind that a definite knowledge of the various factors involved can be obtained, and the variation in results harmonized.

The problems before us then are: (1) to determine the definite relation between the water content of seeds exposed to high temperatures and their viability; and (2) to explain the different degrees of resistance of seeds exposed to high temperatures when treated by different methods.

Materials and Methods

The seeds used in these experiments were Icicle, Black Spanish Winter and Crystal Forcing radish obtained from Henry A. Dreer, Philadelphia. These were selected with the view of comparing the relative resistance of varieties adapted to widely different cultural conditions. The seeds were of good quality and throughout the experiments tested at approximately 90 percent. They were stored in glass-stoppered bottles and kept in a room where the humidity of the air varied but little; the moisture (4 percent) in the seeds remained practically constant throughout the experiments. A tin measure was used to obtain random samples from the bottles and no selection was made other than that the seeds with ruptured coats were discarded.

Three methods were used in heating the seeds. (1) The samples were placed in Florence flasks of 100 c.c. capacity, the bottoms of which were sufficiently large to allow each seed to come in direct contact with the glass. The flasks were placed in a wire cage and entirely submerged in a bath containing about thirty-five liters of water previously heated to the desired temperature. This large quantity of water, kept in circulation by a Köhler stirrer, rendered it possible to maintain a temperature constant to within one half of a degree Centigrade. Increase in pressure during the heating was guarded against by providing the containers with corks through which capillary tubes thirty centimeters long were passed. Temperatures above the boiling point of water were obtained by adding the requisite amount of calcium chloride to the water of the bath. In these latter experiments provision was made for replacing the water evaporated from the bath while the experiments were in progress. (2) The samples in shallow open pans were placed in a double-walled copper oven filled with glycerine. The temperature was controlled to within one half of one degree Centigrade. (3) The samples were enclosed in muslin bags and immersed directly in the water of a bath previously heated to the desired temperature.

The water content of the stored seeds was determined by finding the loss in weight of a random sample brought to constant weight in an oven maintained at 104° C. In the experiments requiring a reduction of the water content of air-dry seeds, the amount of water present was diminished by heating, first in an oven at 60° C. and later in one at 100° C. Samples were treated with the desired temperature when, upon weighing, it was found that the water content of the seeds had been reduced to the desired amount. To increase the water content the air-dry seeds were exposed to a saturated atmosphere until they had absorbed the amount desired in a particular experiment. This method proved satisfactory when only a slight increase in water content of the seeds was desired. When a considerable increase was necessary, the air-dry seeds were soaked in tap water at 20° C. until they had absorbed the required amount. The seeds, superficially dried between towels, were placed in flasks and allowed to stand for a time to permit the water to penetrate uniformly before heating. A momentary immersion of the dry seeds in 95 percent alcohol was found beneficial in that it allowed a quick and uniform wetting of the coats when the seeds were placed in water.

The seeds were germinated on plaster of Paris blocks twelve centimeters square and three centimeters thick. The surfaces of these blocks were crosschanneled so that there were one hundred intersections suitable for the hundred seeds used in each test. The blocks containing the seeds were placed in fiber tubs and water added to a depth of two centimeters. The tubs were covered and kept in a dark room at approximately 23° C. A daily record of the number of germinated seeds was made, and this continued for fourteen days.

Experiments and Discussion

All the earlier investigations in this field were carried on with very dissimilar seeds of widely different, and in no case definitely determined, water content. In no instance was an attempt made to find the effect of high temperatures acting for definite periods of time upon series of like seeds of different but known water contents.

Radish seeds (Icicle, Black Spanish Winter, and Crystal Forcing), with an initial water content of from 4 percent to 71 percent as indicated in Tables I, II, and III, were placed in the flasks already described and heated for thirty minutes at temperatures (50° C. to 125° C.) indicated at the head of the tables. After heating for this period they were placed on the blocks for germination. The results

THE EFFECT OF HIGH TEMPERATURES UPON RADISH SEEDS OF KNOWN INITIAL WATER CONTENT

Table I. Icicle Radish

Temperatures Employed......	50°	55°	60°	65°	70°	75°	80°	85°	90°	95°	100°	105°	110°	115°	120°	123°	125°	Check
Percent of germination:																		
With 71% water present	80%	45	0															87
“ 50% “ “	83%	50	0															88
“ 45% “ “	84%	69	0															87
“ 38% “ “	87%	72	14	0														89
“ 30% “ “		74	25	0														90
“ 23% “ “		74	38	1	0													90
“ 18% “ “		78	66	38	11	0												88
“ 14% “ “		85	74	69	63	26	18	6	0									89
“ 9% “ “		88	82	85	74	54	36	15	10	0								90
“ 4% “ “			86	91	90	91	82	79	76	40	0	0						89
“ 2.3% “ “						89	88	87	90	87	80	72	57	5	0			88
“ 1.3% “ “								90	89	88	82	73	67	32	4	0		89
“ .8% “ “										89	88	85	76	62	32	0	0	89
“ .4% “ “											90	86	78	64	28	14	0	90

Table II. Black Spanish Winter Radish

Temperatures Employed......	50°	55°	60°	65°	70°	75°	80°	85°	90°	95°	100°							Check
Percent of germination:																		
With 71% water present	67%	43	0															88
“ 50% “ “	69%	52	0															90
“ 45% “ “	80%	53	0															90
“ 38% “ “	85%	56	7	0														87
“ 30% “ “		62	46	0														87
“ 23% “ “		73	67	10	0	0												90
“ 18% “ “		76	78	37	21	1	0	0										88
“ 14% “ “		85	86	78	49	37	33	26	0	0								90
“ 9% “ “		87	88	86	67	55	50	38	7	0	0							88
“ 4% “ “			90	90	87	88	82	77	66	38	0							89

Table III. Dreer's Crystal Forcing Radish

Temperatures Employed......	50°	55°	60°	65°	70°	75°	80°	85°	90°	95°	100°							Check
Percent of germination:																		
With 71% water present	87%	60	0															93
“ 50% “ “	89%	66	0															92
“ 45% “ “	92%	66	0															90
“ 38% “ “	93%	71	1	0														90
“ 30% “ “		75	24	0														89
“ 23% “ “		83	74	26	0	0												90
“ 18% “ “		85	81	66	19	10	0	0	0									89
“ 14% “ “		91	84	80	68	47	39	13	3	0								92
“ 9% “ “		91	93	91	83	74	64	56	28	0	0							89
“ 4% “ “			91	90	91	94	88	86	74	25	0							88

indicated in the tables are based upon the treatment of approximately sixty thousand radish seeds. The resistance of the three varieties proved to be so similar that a separate discussion of each is unnecessary.

An examination of these tables shows that there is a definite relation between the initial water content of seeds heated at high temperatures and their viability. Seeds of an initial water content of 71 percent, 50 percent and 45 percent are killed at 60° C. As the water content decreases in the successive series we find the percent of germination to increase and the death point of the sample to be markedly raised. For example as the water content is decreased from 45 percent to 30 percent the lethal temperature shifts from 60° C. to 65° C. Air-dry seeds of approximately 4 percent water content give a normal germination after treating at 75° C. and are killed between 95° C. and 100° C. On the other hand, samples carefully dried until only .4 percent of water is present at the time of treatment, give a normal germination at 100° C. and are killed between 123° C. and 125° C. We find then as the water content increases from .4 percent to 45 percent that the maximum temperature at which a normal percent of germination takes place drops from 100° C. to below 50° C. and that the lethal temperature drops from between 123°–125° C. to 55°–60° C. The resistance of radish seeds exposed to high temperatures decreases as their initial water content increases. Furthermore at temperatures high enough to be injurious, the viability of radish seeds of a definite initial water content decreases as the temperature to which they are exposed is raised.

In recording the percent of daily germination not included in the above tables, a greater or less degree of retardation in the germination of seeds, treated at temperatures a few degrees below their maximum, occurred so constantly that it was thought advisable to make a definite quantitative study of the same. In Table IV are recorded the results of a series of experiments on the rate of germination as affected by initial water content and high temperatures.

Samples of five hundred radish seeds containing 4 percent, 9 percent, 14 percent, and 18 percent of water, respectively, were heated for thirty minutes in 100 c.c. flasks submerged in a bath held at 80° C. and subsequently allowed to germinate on plaster of Paris blocks. The data (Table I) show that at this temperature seeds containing the above water contents suffer in direct proportion to the quantity of water present. The observations were carried on over a period of seven days and the results obtained recorded as indicated in Table IV. The daily percent of germination of the five hundred untreated seeds is shown in the last column. In the other columns is recorded the germination of seeds of an initial water content of 4 percent, 9 percent, 14 percent, and 18 percent respectively, when heated at 80° C. for thirty minutes.

TABLE IV

The Retardation in Germination Caused by Varying Water Content at Temperature of 80° for 30 minutes

Water Content of Seeds when Heated	4%	9%	14%	18%	Check Untreated
Percent of germination the 1st day	6%	.6	0	0	30
" " " " 2d "	21%	5.4	0	0	45
" " " " 3d "	35%	17	7.4	0	14
" " " " 4th "	17%	24.2	11	0	3
" " " " 5th "	3%	10	6	0	2
" " " " 6th "	1%	3.6	2.2	0	1
" " " " 7th "	0%	0	.4	0	0
Total percent of germination	83%	60.8	27.0	0	95

The maximum daily percent of germination in the check (45 percent) occurred on the second day, while in the treated seeds the highest number of seeds germinated on the third or fourth day. Seventy-five percent of the untreated seeds germinated in the first two days as compared with twenty-seven percent for those of 4 percent moisture, six percent for those of 9 percent moisture and zero percent for those of 14 percent moisture. The resulting injury due to the increased water content is shown by the rapid decrease in total germination of the samples, namely: 95 percent, 83 percent, 60.8 percent, 27 percent and 0 percent respectively. The retardation in the germination of radish seeds becomes greater as the injury due to the treatment becomes more marked.

To correlate the results found in the literature with my own, it became necessary to repeat, under control conditions, the different methods heretofore used. In the preceding section (Tables I, II, III) the intimate relation between initial water content and viability at high temperatures was definitely determined, and the suggestion made that the wide difference in the lethal temperature of seeds of similar kinds as reported by different investigators was to be sought in the initial water content of the seeds or in the method employed that would allow an increase or decrease of this water content during the heating process.

Three series of similar samples of radish seeds containing an initial water content of 19 percent were heated at temperatures from 45° C. to 105° C. for a period of thirty minutes (Table V). The samples of Series 1 were heated directly in water, those of Series 2 in 100 c.c. flasks immersed in water, and those of Series 3 in an oven. The seeds were placed upon the plaster blocks for germination. The results in percent of germination are recorded in Table V.

TABLE V

The Effect of Different Methods of Heating upon the Germination of Seeds

Temperatures Employed	45°	50°	55°	60°	65°	70°	75°	80°	85°	90°	95°	100°	105°
Percent of germination of seeds													
When heated in water. Series 1	88%	80	58	2	0	0							
When heated in flasks. Series 2		89	87	81	66	18	9	0	0				
When heated in oven. Series 3							89	88	84	76	60	6	0
Checks, untreated	88%	90	89	88	89	91	90	90	89	88	90	90	89

The results as given in this table show very clearly that radish seeds of similar water content when heated as indicated in Series 1, 2, and 3, respectively, exhibit very different degrees of resistance. The samples heated directly in water suffered a loss at 50° C. and were killed at 65° C.; those heated in flasks suffered a similar loss (approximately 8 percent) at 60° C. and were killed at 80° C.; those heated in the oven suffered a loss of 85° C. (5 percent) and were killed at 105° C. Further it is to be noted that in Series 1 the effects of the temperature are distinctly manifested at 50° C. and that from this temperature the viability decreases very rapidly, extending through a range of only 15° C. In Series 2 similar effects are noted 10° C. higher, namely 60° C., and the viability decreases less rapidly, namely extending through a range of 20° C. The most marked effect is shown in Series 3. The effects of the treatment here lie 35° C. higher than in Series 1 and 25° C. higher than in Series 2. The decrease in viability is slow at first and then very rapid, falling from 60 percent germination at 95° C. to 6 per cent at 100° C.

The data recorded in Table V, Series 2, are practically a repetition of those found in Tables I, II, and III, for an approximately similar initial water content. Starting with an initial water content of 19 percent in Series 1, Table V, we find the injury essentially equivalent to that of seeds with an initial water content of approximately 71 percent (Tables I, II, III). In Series 3, Table V, with the same initial water content, we find the injury essentially the same as that for seeds of 4 percent initial water content (Tables I, II, III). This is exceedingly suggestive.

Three series of weighed samples of radish seeds with an initial water content of 19 percent were heated at 65° C., 80° C. and 95° C. for periods of 3, 6, 10, 15, 22, and 30 minutes respectively. The series were repeated for each of the three methods already indicated in Table V. At the end of each period of heating the samples were removed, weighed, and the loss or gain recorded as indicated in Tables VI, VII, and VIII. The data from these tables were used in constructing the graphs in Fig. 1. The ordinates express the percent of gain or loss in weight of the samples (above or below 19 percent) according as the graph extends above or below the line *AB*. The abscissae represent the periods of time during which the seeds were exposed to the respective temperatures. In order to obtain the actual water content of the seeds, one must add or subtract the indicated

TABLE VI

The Increase in Weight of Seeds when Heated in Water

Periods of Exposure...............	3 Min.	6 Min.	10 Min.	15 Min.	22 Min.	30 Min.
Percent of increase at 65° C....	13%	21	29.4	37	45	53.2
“ “ “ “ 80° C....	20.1%	32.2	45	57.1	60.3	61
“ “ “ “ 95° C....	23.4%	39	51	59.8	60.1	60.4

TABLE VII

The Loss of Weight in Seeds when Heated in Flasks

Periods of Exposure...............	3 Min.	6 Min.	10 Min.	15 Min.	22 Min.	30 Min.
Percent of loss at 65° C........	2%	3	2.9	3	3	2.9
“ “ “ “ 80° C........	4%	4.9	5	5	5.1	5.1
“ “ “ “ 95° C........	7.3%	8	7.9	7.9	8	8

TABLE VIII

The Loss in Weight of Seeds when Heated in an Oven

Periods of Exposure...............	3 Min.	6 Min.	10 Min.	15 Min.	22 Min.	30 Min.
Percent of loss at 65° C........	3%	5	6.3	7.9	9.8	10.5
“ “ “ “ 80° C........	4%	6.3	8	10	12.1	13
“ “ “ “ 95° C........	5.3%	8	10.1	12.4	14.4	15.9

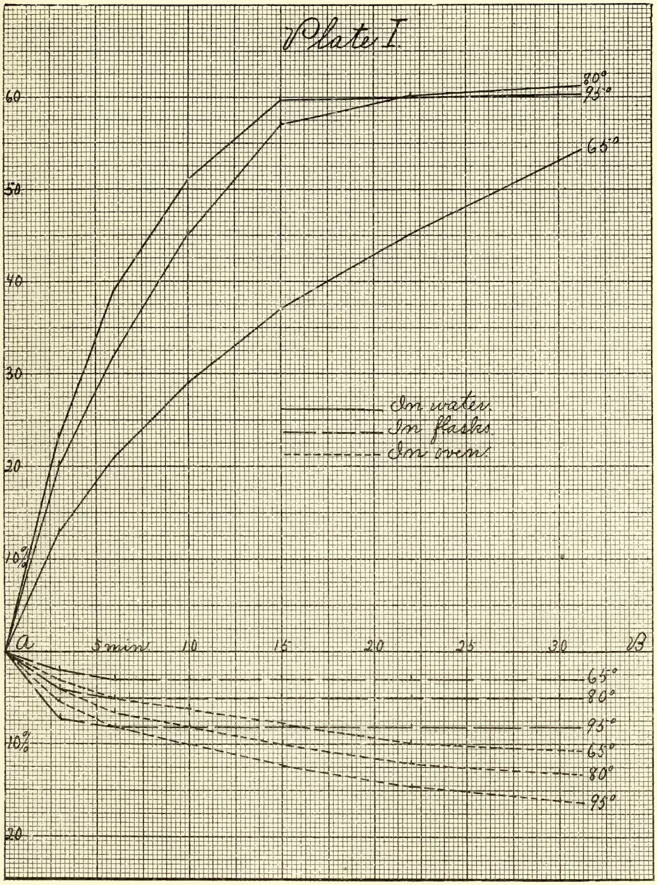

FIG. 1. Graphs showing the changes in water content obtained by heating radish seeds.

gain or loss (Fig. 1) to or from the initial water content (19 percent) of the seeds used.

These graphs show the striking changes in water content obtained by heating radish seeds of 19 percent initial water content by the three different methods. (A subsequent experiment will show that the gain in weight of the samples heated by method 1 does not express accurately the amount of water absorbed.) The seeds heated directly in water increased in weight very rapidly; those heated in the flasks decreased in weight for about six minutes and then remained constant during the remainder of the heating; those heated in the oven gradually decreased in weight throughout the entire thirty minute period of treatment. At the end of thirty minutes the seeds heated at 95° C. were found to have a water content for method 1, of 79 percent, method 2, 11 percent, and method 3, 3 percent. A similar variation, though less extensive, is seen in seeds heated at 80° C. or 65° C. respectively (Fig. 1). It has been shown that the resistance of seeds exposed to high temperatures is inversely proportional to the initial water content at the time of heating. The results obtained in the above experiments (Fig. 1 and Table V) are in entire accordance with this statement.

Data are not at hand upon which to base a complete explanation of the resistance of radish seeds to high temperatures. Seeds heated in air lose water by evaporation which is a cooling process. Moreover, air and water differ materially in their ability to transmit heat. Hence we cannot say with absolute certainty that we have subjected the seeds used in the corresponding tests to exactly the same temperatures for the same periods of time. Data from seeds heated at a given temperature without suffering any change in the water content during heating would be desirable. However the results as shown in Table V between water- and oven-heated seeds cannot be explained on the basis of cooling. These questions together with related ones will form the basis for a future investigation.

The rate of gain or loss in water content of the seeds when treated by method 1, 2 or 3 is of interest, since, owing to a change in their water content, the seeds show a low (Series 1) or a high (Series 3) resistance. The rapid absorption of water in the seeds of Series 1 concomitant with the high temperature is responsible for the marked injury as indicated in Tables I, II, III, and V. On the other hand the rapid loss of water in the first six minutes of treatment in Series 2 and the subsequent maintenance of approximately the same water content for the whole period of heating—due to equilibrium of vapor tension—brings the resistance markedly higher. In the seeds of Series 3 the water vapor was constantly carried off and consequently the seeds lost water throughout the process.

Attention should be called to the fact that radish seeds heated directly in water lose in dry substance. It is necessary to know the extent of this loss in order to interpret more accurately the results found in Table VI. This experiment was repeated and the loss of dry matter determined and recorded as shown in Table IX.

Table IX

The Loss in Dry Weight of Seeds when Heated in Water at 65° C., 80° C., and 95° C. Respectively

Periods of Exposure	3 Min.	6 Min.	10 Min.	15 Min.	22 Min.	30 Min.
Percent of loss at 65° C.	.21%	.4	.7	1	1.4	2
“ “ “ “ 80° C.	.39%	.82	1.4	2.1	3.6	5.4
“ “ “ “ 95° C.	.78%	1.6	2.6	4	6.1	7.9

These results show that there is a gradual loss in dry weight when radish seeds are heated directly in water at the temperatures indicated. Moreover the higher the temperature of the water the more rapid is the loss in weight. The seeds heated at 65° C., 80° C., and 95° C. for thirty minutes lost 2 percent, 5.4 percent, and 7.9 percent respectively. It follows from this that the actual amount of water taken up by radish seeds heated directly in water is considerably more than the increase in weight as indicated in Table VI.

Conclusions

The resistance of seeds of *Raphinus sativus* L. exposed to high temperatures is inversely proportional to the initial water content of the seeds at the time of heating.

At temperatures high enough to be injurious the viability of radish seeds of a given initial water content decreases as the temperature to which they are subjected is raised.

The general resistance of Icicle, Black Spanish Winter and Crystal Forcing radish seeds exposed to high temperatures is very similar.

Radish seeds injured by high water content and high temperatures are retarded in their germination. This retardation becomes more marked as the temperature or water content or both is increased.

Radish seeds of the same initial water content show very great differences in resistance when heated at the same temperature but by different methods, namely: in water, in dry corked flasks, or in open dishes in ovens.

The amount of water absorbed or given off by radish seeds during treatment is the chief factor determining the resistance of the seeds heated at the same temperature by the different methods.

When radish seeds are heated directly in water they suffer a gradual loss of dry substance. This loss becomes greater as the temperature of the water is increased.

University of Illinois,
Urbana

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CONTENTS

The taxonomy of the Agaricaceae ..... William A. Murrill 315
Observations on forest tree rusts.
James R. Weir and Ernest E. Hubert 327
Endothia pigments. I ..... Lon A. Hawkins and Neil E. Stevens 336
Observations on an Achlya lacking sexual reproduction ..... Wm. H. Weston 354
The Fritillaria rusts of Europe and America ..... Charles C. Rees 368

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THE TAXONOMY OF THE AGARICACEAE*

WILLIAM A. MURRILL

No classification is perfect; nature recognizes no very marked divisions. As Professor Massee, of Kew, used to say, "Why make a fence? Some rooster is sure to get on top of it with his head on one side and his tail on the other." And often the higher realms of perfection are of little practical use. It is vastly more important to help twenty students to a better knowledge of a group of plants than to tickle the fancy and win the praise of one who no longer needs help. Any one leaving the beaten track is subject to criticism, when he should get only sympathy.

Genera are not now considered sacred entities, but simply groups of species that are more closely related to each other than they are to other groups. New species are usually forced on one; and they entail a lot of extra work requiring initiative, insight, and independent thinking. Examine the shelves of almost any taxonomist and you will find them cluttered up with species he knows are new and is too indolent to describe. The very men who criticize most loudly the multiplication of species would probably lack the courage, ability, and patience to publish new ones.

Nomenclature and the rules of nomenclature are only a means to an end, like the rules of a whist game. No matter how meritorious one's work may be, tradition and custom are as hard as granite rock and the older workers can hardly be expected to change the names to which they have become accustomed. As our knowledge grows, forms of widely distributed species may merge into one and names may be reduced to synonymy. In such cases, it is very unwise and unscientific

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to uphold an error because it happens to be one's own. The injection of the personal element only impedes progress; the mind should be kept open and unbiased, ready for any new light. What crimes have been committed, both in Europe and in this country, in the name of Science! Crimes of ignorance, of carelessness, of egotism, of petty selfishness, of border antagonisms and national hates! But that is human nature, and, after all, the scientists have more than their share of piety.

Morphology and Taxonomy

Not so very long ago, most American botanists were taxonomists. Now the pendulum has swung the other way. The temptation is very strong for professors in small colleges to limit themselves to a few forms and to go rather thoroughly into their structure and life cycle rather than to attempt to know and classify many forms, which requires a large herbarium and a large library. National and state aid, also, has been largely in the direction of physiology, morphology, and pathology, which is only natural and proper; but it means that taxonomy and the study of plants not strictly economic must be emphasized in institutions properly endowed and equipped for this purpose and that all botanists who enjoy the advantages of such institutions should use them to the fullest extent if the proper balance in the study and teaching of botany is to be maintained.

The old quarrel between the taxonomist and the morphologist is based partly on a lack of sympathy due to ignorance; and one way to restore friendly relations is to increase the breadth of view on both sides. The taxonomic laboratory should really be an "assembly room" for all kinds of information about plants, derived from the geologist, the phytogeographer, the morphologist, and the physiologist. No fact should be overlooked, no source of information should be ignored.

The object of the taxonomist should be not only to arrange his specimens in an orderly way in the herbarium, but to gain the greatest possible knowledge concerning the species; using all the specimens at hand, all notes from various collections, and all that has been written about the species and its distribution, as well as its habitat, abundance, variation, and economic bearing. Such a study is not easy, but involves the highest mental processes. The weighing of all the facts and observations regarding species and their systematic position often taxes the best judgment. It is easy to collect specimens as a fad; it is not difficult to know a great deal about the objects of nature; but few are capable of pursuing the study of a group until it is rounded out into a perfect and orderly whole, so that others may easily follow and understand. When such a work is done, it is the simplest thing in the world to criticize both the methods and results. It is the object of all devoted nature lovers to add as much as possible to the sum total of natural knowledge. No part of nature is too humble to demand our best endeavors when it is considered in relation to the numberless objects that make up the great universe, and it often happens that what seems very small and unimportant may hold the secret key to something that we look upon as vastly important.

So much for the taxonomist. Let us next see what the isolated worker or professor in a small college can do toward broadening his botanical outlook.

1. He can make sure that he knows by reading or otherwise something of the relatives and relationships of every form he studies morphologically, cytologically, physiologically, or pathologically. This would be a distinct advance. For example, a student investigating the cytology of the scrub pine could learn something of the various species of pines and their near relatives, and a student tracing the life history of a certain species of Gymnosporangium could learn to recognize another species of the same genus in case he happened upon it accidentally. It is just as disgraceful for a morphologist not to know the taxonomy of a type he is investigating as it is for a taxonomist not to know why sap rises or the significance of reduction in chromosomes.

2. He can begin, if he never completes it, a local flora of his region, including all groups of plants. Some groups offer excellent opportunities for field study even during the winter, while many of the lower forms are less difficult than the flowering plants. By preserving specimens, taking good notes, and securing photographs or drawings, this work may be made really valuable. There is hardly a locality in America that does not need careful botanical work of this kind. Here in New York City, we have only made a beginning. With taxonomic botanists becoming scarcer every year, it will be a long, long time before we have any adequate knowledge of the flora of this great country. At present, our books only emphasize our ignorance and the gaps in our herbaria remind one of a child shedding his milk teeth. The morphologist could do most of his taxonomic work while out for exercise or on vacation, and it would only give him zest for his more special problems.

3. He can have a compact and easily accessible synoptic collection representing the principal groups of plants, with a few common and characteristic species in each group, so that he and his friends or pupils may obtain some idea of the extent and variety of the plant kingdom. When one takes a tramp or travels, he sees plants, and often these are about the only living things he does see. Now, it would seem hardly fair to one's intelligence to disregard all this wealth of interesting material and be content to go through life both ignorant of it and uninterested in it.

A speaking acquaintance with plants is obtained by observing their form, color, habits, and relationships, rather than by dissecting them. To begin with the microscope is to begin at the wrong end. Let us not be too proud to know the names of the common flowers that bloom at our feet. It is Nature's way with the child; and it is the logical method for the learner in any subject.

Collecting the Fleshy Fungi

The fleshy fungi, on account of their perishable nature, present many difficulties to the collector, and I have found it almost impossible to secure good specimens without going after them myself. Whenever and wherever fleshy fungi are collected, the following ideal for the herbarium specimen should be kept in mind:

1. Ample typical specimens in all stages, well dried and well preserved without pressing. Specimens in fluid have little value.
2. Full descriptive field notes, especially of perishable characters.
3. A colored sketch as accurate and detailed as possible and a photograph if practicable. Color notes with a color guide at hand may be used with the photograph.

In collecting, it is possible to attempt the entire group of fungi but this is seldom successful. One gets accustomed to looking for certain forms in certain places and it is quite difficult to train the eye to several different sets of conditions. When one collects leaf-spot fungi, he goes more or less into the open and carries a plant-press or vasculum; but when one seeks fleshy fungi, he usually goes into the woods and carries a basket. From the standpoint of collecting pure and simple, the fungi may be divided into three classes: (1) those occurring on leaves, in which the host is pressed just as in flowering plants, (2) those occurring in various places, but requiring no special care in drying for preservation. This class includes the molds, many of the ascomycetes, all the lichens, practically all the polypores, most of the gasteromycetes, all the Thelephoraceae, most of the Hydnaceae, and many of the smaller and tougher gill-fungi, (3) those requiring the sun or artificial heat for drying, this class including practically all the fleshy gill-fungi, many of the larger fleshy cup-fungi, a few of the gasteromycetes, some of the coral-fungi, hedgehog fungi, and polypores, and all the Boletaceae, this last family being the most difficult for the collector.

It is important that fleshy specimens be allowed to dry as naturally as possible, even if artificial heat is used, since they often assume characteristic shapes in drying. The heat should never be strong enough to cook the specimens, and they should not be pressed in order to mount them in packets. A fresh specimen badly infested with insects may be treated with naphthalene or chloroform in order to prevent the destruction of the specimen before it can be dried. In the mountains of Austria and Italy, where the air is unusually dry, I found artificial heat rarely necessary. In the wilds of Maine and Cuba, I used a special drying oven over a camp stove; at the Lake Placid Club in the Adirondacks, a sunny, steam-heated room; in the Catskills, a large open fireplace; in Oregon, a "biplane" made of window screens suspended over a wood stove; in Virginia, a garret over the kitchen stove; on the Vanderbilt estate in North Carolina, the ordinary sunshine; and in Jamaica and Mexico, a drying oven over two tin oil lamps, which were often kept going all night.

It sometimes happens that a botanist may know a distant region better than the one in which he lives. When one goes away for a definite object, he can devote his time to that object, while at home his attention is often absorbed with numerous other interests. In my own case, while I have been able to get a fair knowledge of the species occurring in the immediate vicinity by getting up at an early hour in the morning, I have given rather special attention to northern New York, Maine, Virginia, North Carolina, Washington, California, Mexico, Jamaica, Cuba, and many parts of Europe, the fungi brought back from these regions amounting to over 30,000 herbarium specimens.

The mycological herbarium at the New York Botanical Garden contains about 200,000 specimens, half of which were obtained from Mr. J. B. Ellis, who at the time of his death had described more new species than all other American mycologists together. The value of such a collection can hardly be overestimated. From a purely botanical standpoint, it is highly important that original and representative specimens of all groups of plants be thus preserved for purposes of reference and comparison; and, since questions of origin, distribution, and variation always enter into studies of classification, it is desirable to have these collections as complete as possible. From the standpoint of applied botany, the vast number of destructive plant diseases caused by fungi relate this subject very intimately with horticulture, agriculture, forestry, and allied sciences.

Aside from the use of the collection by systematic botanists, plant pathologists, and foresters, there is a large and increasing interest manifested in fungi by the plant-loving public, drawn by fondness for the queer and unknown, or attracted by bright colors and peculiar forms, or by their extensive use as food. To all these, the collection affords the keenest pleasure and offers opportunities for further knowledge and enjoyment.

This collection of fungi is to be the basis of nine volumes of *North American Flora*. As the various groups are worked over and new species published, the number of type specimens in the herbarium will be greatly increased. Students, collectors, and investigators throughout the country will continue to send in specimens for determination and comparison, and will come here to consult not only the originals, but the array of additional specimens that show the variation and the geographical distribution of given species and groups of species.

It is hoped that important contributions may in time be made to questions of geographical distribution on the basis of various collections from distinct regions. For the purpose of recording the distribution of species conveniently and quickly, a chart has been prepared, copies of which may be properly marked and pasted on the inside of the species covers, to show at a glance just where a particular species is known to occur.

If one wishes to distinguish specimens from different regions in the herbarium, he may use gummed paper markers of different colors on the species covers, or simply indicate the regions by numbers or letters, as shown in the following table:

I.	North America	Na	White	VI.	India	In	Orange
II.	Tropical America	Ta	Red	VII.	China and Japan	Cj	Yellow
III.	South America	Sa	Blue	VIII.	Malaya	Ma	Brown
IV.	Europe and Siberia	Es	Gray	IX.	Australia	Au	Pink
V.	Africa	Af	Black	X.	Islands	Is	Green

The Mechanical Side of the Herbarium

The mechanical side of the fungous herbarium presents fully as many difficulties as the preparation of specimens in the field. Some of the specimens are small and flat, while others are large and bulky; some are tough or woody, others are fragile; some may be poisoned once for all, while others require constant attention to prevent their destruction by insects.

The model fungous herbarium contains all the specimens of a group in a single series and is at the same time neat and easily consulted. Let us begin with the ordinary herbarium sheet to which is attached envelopes of various sizes containing the specimens. It is never desirable to leave the specimens exposed on the sheet as in flowering plants, although this old method had obvious advantages. In case of small specimens that might be lost, they are best enclosed in pill-boxes or small elongated paper boxes, or in open cradles with cardboard bottom and sides of cypress or cork strips attached with glue. Such containers should always be placed within the envelopes before mounting. A very convenient paper box is made with a loose cover so as to avoid delay in opening it. The more specimens that are fastened to sheets, the less trouble there will be.

For specimens too large to fasten in this way to sheets, boxes of various sizes will have to be used and these should be either glued to good cardboard, only one species to a card, or placed in a light wooden tray that fits the pigeon-hole. In order to prevent the great waste of time incident to examining a large amount of material and in opening boxes or packets, a set of sample specimens may be mounted in uniform boxes fastened to cardboard and covered with transparent tops made of gelatin or celluloid. With a set of these samples, hundreds of specimens may be consulted and compared in a few minutes. Such an arrangement is peculiarly adapted to universities and small herbaria where distribution is not so much an object as is the determination of specimens. There still remains the odd lot of boxes too few to mount on cardboard which can only be placed in a wooden tray and listed on the outside of the tray. Such individual representatives of different species cannot be distributed at once through the herbarium, but must wait until additional material allows the use of the cardboard.

The preservation of fungi against insects has always been a difficult problem for the curator. Many methods have been tried in various herbaria without complete success. Carbon bisulfid has been mainly used in this country, but the results are not satisfactory. Corrosive sublimate, so extensively employed for flowering plants, is not only valueless but decidedly harmful to many of the higher fungi, since it alters or destroys their surface characters and often changes their substance to a marked degree. It is much better to lose some specimens than to have the whole collection thus altered. In the case of large woody specimens, also, it is very difficult to secure sufficient penetration to preserve the interior portions.

The substance I have used with great success is naphthalene flake, of the best quality. Experiments conducted here have shown that adult insects are killed in a few hours when placed in a box with this substance, and it is probable that those emerging from the pupa stage succumb in less time. Specimens are treated when first obtained, and those peculiarly susceptible are kept in an atmosphere of naphthalene more or less all the time. In going through the collections, when a packet or box is found containing insects, a spoonful or more of naphthalene is added and the incident closed. Possibly there are insects not yet acquired or some that do not thrive in this region that are not amenable to this treatment, but it has been more satisfactory here so far than any other method I have seen tried.

All fungi found upon leaves are treated with corrosive sublimate. This is done chiefly to preserve the leaves intact, the fungi being so small that, with few exceptions, insects would hardly do them serious damage. All other fungi, particularly the conspicuous forms known as mushrooms, bracket fungi, etc., are placed in boxes with naphthalene flake for several weeks or longer, according to the season, before distributing them in the herbarium. Groups peculiarly liable to attack are examined once or twice a year and fresh naphthalene added when necessary. After a box collection has been once cleared of pests, it is not so difficult to keep them out, with a fair amount of precaution and vigilance.

The Arrangement of Illustrations

The ideal herbarium contains specimens arranged in a single series, with all notes and illustrations classified with the specimens. The maintenance of more than one series is both a complication and an aggravation. However, throughout most of Europe, illustrations are kept in a separate series, just as exsiccati usually are; and I might remark that both exsiccati and illustrations should be in duplicate so that one set may be distributed with the specimens and the other set kept on file for ready reference. At Kew, a splendid set of portfolios has recently been made to hold the large number of colored illustrations made by Cooke, Massee, and others. In the Fries herbarium at Upsala, the drawings are mounted on cardboard and kept in a separate case. The advantage of having colored illustrations readily available when fresh specimens are brought in for comparison appears at once, since characters are then used for determination which disappear when the specimens are dried. Herbarium specimens are rarely consulted for comparison except with dried material. It may also be desirable to know whether there exists in the collection a good illustration of the specimen in question so that steps may be taken to fill the gap as new collections are brought in. It has been decided to adopt the following arrangement with our collection of fleshy fungi:

1. Keep a set of colored drawings and photographs convenient for ready reference.
2. Keep all other drawings, such as those of sections, spores, etc., with the specimens in the herbarium; and prepare a duplicate set of photographs and colored drawings for the herbarium whenever practicable.

Water-color paintings should be kept in a perfectly dry, dark place. Naphthalene, camphor, and carbon bisulfid are not particularly harmful to water-colors, but sulfur dioxid, hydrogen sulfid, and fumes of ammonia or acids should be carefully guarded against. The colors used should be the best and most permanent on the market, and each color should be actually tested by the artist if possible before it is used.

THE NEED OF AN AMERICAN ILLUSTRATED WORK

While on the subject of color, I wish to remark that one of the greatest needs of mycology in this country is a comprehensive illustrated work on the larger fungi. The various countries of Europe are well supplied with such works, some of them quite old and very elaborate. Had it not been for these books, the work of many mycologists would have been practically lost or left in such a state as to be more or less useless. America has nothing to compare with any of the illustrated works on fungi in Europe. The need of such a work is fully realized by all; but it would require not only a well-equipped herbarium and library, but also a considerable amount of money, probably

$50,000, to carry out such an undertaking. Artists would have to paint the plants in the fresh condition where they grow and this would necessitate reaching various parts of the country during the growing season, although a large beginning might be made in any given locality. The plates should be prepared and reproduced in the best possible manner, and accompanied by accurate and comprehensive descriptive text. Such a work would be useful to the forester who wishes to protect his trees from wood-destroying fungi, to the collector of edible mushrooms who wishes to use them for food and to guard against poisonous species, to the student in college or university, and to the general nature-lover in whatever part of the country he might live. There is nothing that would give a greater impetus to the study of fungi in all parts of North America than the publication of such a great illustrated work.

The Classification of the Gill-fungi

Coming now to a discussion of the taxonomy of the Agaricaceae in a more limited sense, the history of mycology in Europe shows that some of the older men, like Schaeffer and Bulliard, devoted their attention to describing and illustrating *species* and thought very little about *genera;* while others, like Persoon, Roussel, Gray, and Fries, attempted to improve upon the rather primitive divisions of Linnaeus. Then came the general adoption of the Friesian classification through the publication and wide distribution of the *Systema* and Saccardo's *Sylloge,* followed by demands for improvement from Quélet and Patouillard in France, Karsten in Finland, Hennings in Germany, and Underwood and Earle in America.

When one travels from country to country and from one herbarium to another, he gets accustomed to changing his nomenclature as he does his language and his money. The claim of "existing usage" put forward by some has no value whatever unless it refers to the names used in Saccardo's work, which is merely a convenient, though disorderly, compilation of species as they are published, arranged according to a system in vogue when the work was started many decades ago. Karsten, a pupil of Fries, questioned the latter's classification because based on too few characters. Patouillard and Fayod considered microscopic characters of great importance, while Maire goes so far as to include cytological characters.

What we need in America is a classification that is impartial, practical, and modern, including all the improvements possible and based on the study of American rather than European material. We want no "pounds, shillings, and pence" in the form of cumbersome subgenera, subsections, and subspecies. The taxonomist must know but not recognize varieties, which are essential to the gardener, the physiologist, and the plant breeder. Let the grouping be as natural as possible, but artificial when conducive to clearness. The absence of sex in the gill-fungi gives one considerable liberty and our knowledge is still far from complete. A system of classification representing the genetic relationships of the higher fungi is hardly yet in sight. If the species could be collected and grown together under cultivation, the glad day might be hastened, but they cannot. Every house has a garret; so has every family a genus or two which catch everything not wanted elsewhere. Do not be too particular with the misfits, but do not throw them out of the window; they may fit in when you move to the next house.

In seeking suitable characters for classification, one must use what comes to hand, and the same characters may not be available for different groups. The best and most constant primary character for the gill-fungi seems to be the color of the spores. Earle tried to use the "partial veil" but failed on account of its evanescent and variable character. The form and surface markings of the spores may be quite characteristic, as in Entoloma and Inocybe, but other good characters should be associated with them. I believe that Patouillard goes too far with microscopic characters, and, moreover, that these should be used in keys as little as possible, in order to save time and trouble. A key character need not necessarily be the most important generic character, but only the most convenient.

Recent researches on color in the flowering plants have shown this character to hang on a very slender thread sometimes in that group, but what would we do in the fleshy fungi without the recognition of color? Poisonous properties alone would hardly seem to be a sufficient basis for the separation of species, and still there might be a good practical reason why they should be recognized in certain instances. I have in mind the variations in the poisonous properties of *Venenarius muscarius*, *V. pantherinus*, and *Chlorophyllum molybdites*, and the separation of *Clitocybe sudorifica*, a poisonous species or variety, from *Clitocybe dealbata*, generally considered harmless but not differing morphologically from *C. sudorifica*. Ordinarily, physiological properties would seem to have no taxonomic standing, but they might be suggestive and lead to more careful morphologic research.

The many changes made in generic and specific names are to be deplored, but they are unavoidable. As already intimated, the systems of classification in vogue in Europe were not in harmony and were based on different conceptions from ours, so that they had to be worked over and adapted to our needs. The American code of nomenclature adopted for *North American Flora* over a decade ago has been found to work remarkably well and we see no reason to change it, even if such a thing were possible, for the set of compromise rules recently formulated which will never be consistently followed anywhere in the world. People ask me why I take up *Melanoleuca* for dear old *Tricholoma*, not knowing that Bentham used *Tricholoma* for a genus of flowering plants as early as 1820. They say it is a shame to discard *Amanita* and use *Venenarius* for our most poisonous mushrooms, little dreaming that in the long ago *Amanita* and *Agaricus* meant the same thing and we could not keep them both. It is not my fault that the old fellows did their work so poorly and with such a delightful disregard of priority rights.

Neither is it my fault that American material has been so poorly determined by European mycologists. They have no more interest in America than we have in the Fiji Islands or in Timbuctoo, and when they receive our specimens they are very apt to be reminded of a similar European species and be satisfied with that. Then, there is the great difficulty in studying dried specimens of fleshy fungi unless one has seen them in the fresh state. Specimens lose something in drying that can never be replaced. That is why I have often sat up half the night over the drying oven when the hunting was good in one of those far-off, wild, and virgin forests "somewhere" in North America or Europe.

I wish now to bring to your attention the system of classification I am using for the gill-fungi. Much time might be devoted to the grouping, the characters, and the descriptive terms employed, but a prolonged discussion of these details would only weary you. I prefer rather to outline briefly the main groups of this family and to illustrate them with colored slides of some of the more common and interesting species.*

New York Botanical Garden.

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* At the conclusion of the paper, lantern slides were used to illustrate the classification of the gill-fungi.

OBSERVATIONS ON FOREST TREE RUSTS

James R. Weir and Ernest E. Hubert

The undertaking of the checking by cultural methods of various forest-tree rusts occurring in the Northwest has established several host relationships previously held doubtful. The recent works of Fraser[1] and Ludwig[2] have aided in the clearing of some of the problems concerned. Fraser's results with species of Uredinopsis on ferns and the final conclusion to the effect that the five species of Uredinopsis used in his experiments have their aecial stage on *Abies balsamea* is an important contribution toward a clearer understanding of the interesting group of rusts occurring on ferns. The five species with which Fraser worked are *Uredinopsis struthiopteridis* Störmer, *U. osmundae* Magn., *U. atkinsonii* Magn., *U. mirabilis* Magn., and *U. phegopteridis* Arth., and a study of the microscopical characters reveals no great differences between them. Fraser[3] in his last article came to the conclusion that all of the five species with which he had been working were identical with the exception of *U. mirabilis* and considered this one different on account of the fact that positive results with aeciospores from *Abies balsamea* were secured on *Onoclea sensibilis* only. In a recent communication received from Fraser, March 27, 1916, he states that Arthur examined all the field collections of *Peridermium balsameum* Pk. and cultures and came to the conclusion that there are no morphological differences in the aecial stages produced on *Abies balsamea* by inoculations of the five species of Uredinopsis. A close comparison of the spore measurements and lengths of beaks of the five species as published by Arthur[4] show no great differences in size from what might be expected as a result of the influences of the various fern hosts. The variations in the spore markings are negligible. In view of the results secured by Fraser and the determinations by Arthur, it is suggested that these five species be combined under the name of one species of Uredinopsis. The aecial stages of all five species have been found to be identical with *Peridermium balsameum* and the close similarity of *P. pseudo-balsameum* (D. & H.) Arth. with *P. balsameum* has led us to consider them here as one species, namely, *Peridermium balsameum.* The differences in the description of the two species do not seem to be of sufficient importance to continue their separation. The description given by Arthur and Kern[5] give a slightly larger spore for *P. balsameum* and no mention is made of the color of the spores of *P. pseudo-balsameum* which are colorless as in the other species. The peridia of both are fairly long (0.75–1 mm.) and with the colorless spores furnish excellent means of identification. All stages of this rust should therefore be referred to one species of Uredinopsis. The aecial stage was not only found in abundance this season on *Abies grandis* but also on *A. lasiocarpa.* This fungus has been collected in the Northwest in 1896 under the name of *P. pseudo-balsameum* (D. & H.) Arth. Hedgcock[6] reports *P. pseudo-balsameum* on *Abies grandis, A. lasiocarpa*, and *A. nobilis* in 1912. In a recent article by Schmitz[7] a claim is made to the first collection of the fungus west of the Mississippi Valley. A glance at the literature[8] will show that *Peridermium balsameum* was collected on *Abies grandis* on the slope of Mt. Paddo, Wash., by W. N. Suksdorf in October, 1903. *P. balsameum* was collected on *Abies grandis* in California in 1896. Many other collections of this fungus have been made since then and the collections of this laboratory at Missoula show considerable material (eight collections) from the northwestern states collected during the years 1911 to 1916.

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[1] Fraser, W. P. (*a*) Cultures of Heteroecious Rusts. Mycologia, 4: 175. 1912.
(*b*) Further Cultures of Heteroecious Rusts. Mycologia, 5: 233. 1913.
(*c*) Notes on *Uredinopsis mirabilis* and Other Rusts. Mycologia 6: 25. 1914.

[2] Ludwig, C. A. Notes on Some North American Rusts with Caeoma-like Sori. Phytopathology 5: 273, 1915.

[3] Fraser, W. P. Notes on *Uredinopsis mirabilis* and Other Rusts. Mycologia 6: 25. 1914.

[4] Arthur, J. S. Uredinales. N. Amer. Flora 7: 115. 1907.

UREDINOPSIS PTERIDIS

During the past two seasons a very interesting Peridermium has been collected on the needles of *Abies grandis* (fig. 1). This fungus is conspicuous by its appearance on the second year needles of its host, by its white aeciospores and unusually long peridia. Most of the other needle rusts occurring on conifers occupy the needles of the season and this fact is accounted for by the overwintering of the telial stage. The infection of these Peridermia is produced in the spring on the youngest needles and the fungi mature the same year. This Peridermium has its alternate stages upon *Pteridium aquilinum pubescens* Underw. (fig. 2) and the conclusion is that the telial stage does not winter over as is the common habit of such rusts. The needles

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[5] Arthur, J. C., and Kern, F. D. Uredinales. N. Amer. Flora **7**: 115. 1907.

[6] Hedgcock, G. G. Notes on Some Western Uredineae Which Attack Forest Trees. Mycologia **4**: 141. 1912.

[7] Schmitz, H. Preliminary Note on the Occurrence of *Peridermium balsameum* in Washington. Phytopathology **6**: 369. 1916.

[8] Arthur, J. C., and Kern, F. D. North American Species of Peridermium. Bull. Torrey Club **33**: 403. 1906.

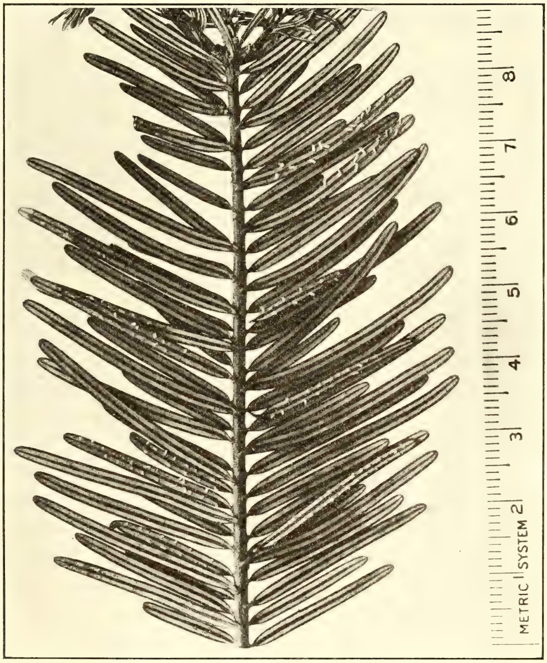

FIG. 1. *Uredinopsis pteridis.* I stage on *Abies grandis* showing appearance upon second year needles.

of the fir must become infected during the same summer or fall in which the telia mature on the fern. Such being the case the advent of lower temperatures prevents the fruiting on the needles until favorable conditions are again present, which is in the early spring of the following year. This is borne out by the collection of the aecial stage fully matured on *Abies grandis* as early as April 12, 1916. This is the earliest collection of any needle rust made in this locality. Other collections were made on April 14, May 2, and June 17, of the same year.

On June 19, 1916, sowings of aeciospores of the above fungus on *Abies grandis* were made on two plants of *Pteridium aquilinum pubescens*. The plants were raised in the greenhouse at Missoula, Mont., from rhizomes dug up in the field on September 4, 1915, and the inoculations were made by the use of celluloid cylinders and cotton plugs. On July 25, 1916, a medium infection of uredinia was found on one of the plants while the other bore no results. The control plants remained normal. A large number (15) of collections of the fungus on *Pteridium aquilinum pubescens* (fig. 2) made throughout Idaho, Washington, and Oregon was always in immediate association with the rust on the needles of Abies. In one particular instance at Lucern Lake, Wash., August 23, 1916, a lake flat was grown up to young *Abies grandis* and the braken fern. The foliage of the latter was completely parasitized by *Uredinopsis pteridis* D. & H. while the needles of the fir were seriously infected with the aecial form of the fungus. No other forest tree rust was present in the vicinity. After a close comparison of the microscopical characters of the above produced uredinial stage with authentic material of *Uredinopsis mirabilis*, *U. osmundae*, *U. struthiopteridis*, *U. atkinsonii*, *U. phegopteridis*, and *U. pteridis*, it was found to coincide with the latter. A careful study of the published descriptions[9] of the species of Uredinopsis in connection with the above culture showed that no great differences existed between *U. copelandi* Sydow and *U. pteridis* other than the hosts. A slight difference in the size of the spores is to be noted. *U. pteridis* has a spore measurement of 11–18 by 30–58 μ and that of *U. copelandi* is 14–18 by 31–40 μ, a difference in length of about 19 μ. This variation is no greater than is found usually occurring in spores of a single species. In comparing the five species of Uredinopsis which have been found to produce an identical aecial stage on *Abies*

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[9] Arthur, J. C. Uredinales. N. Amer. Flora 7: 115. 1907.

balsamea, it is found that the spore measurements vary to as great an extent as 19 μ. It was also found that the spores of the above five species bore a similarity in the length and shape of the beaks, these varying in length from 3–10 μ to 12–26 μ with almost similar spore markings. The other two remaining species of Uredinopsis, *U. pteridis* and *U. copelandi*, both have spores with short stout beaks

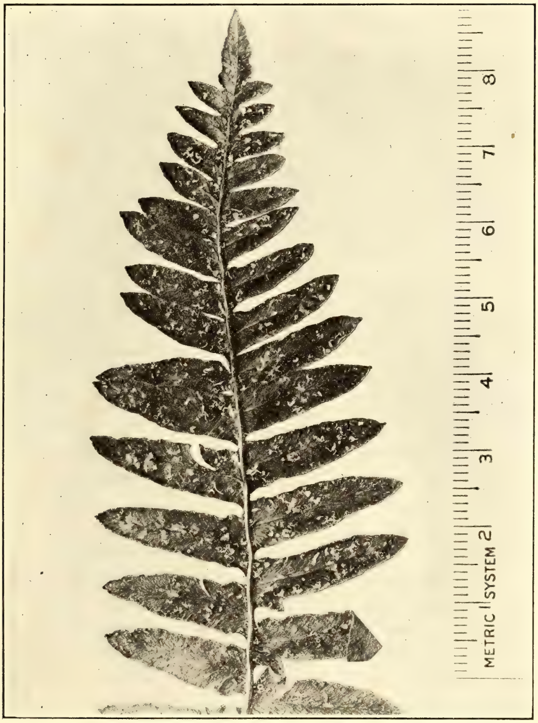

FIG. 2. *Uredinopsis pteridis.* II stage of *Pteridium aquilinum pubescens.* Note the coiled spore masses.

measuring 3–7 μ long. The spore markings of these two are very similar and are very pronounced when compared to the other five species of the genus. Judging from this it seems that no great reliance in respect to identity of species can be placed upon spore measurements alone.

It is suggested, in view of the above comparison, although cultures are necessary for final determination, that *U. copelandi* be considered identical with *U. pteridis* and placed under the latter species. A technical description of the aecial stage of *U. pteridis* follows:

0. Pycnia not found.

I. Aecia from a limited mycelium appearing on second year needles, hypophyllous, not crowded, forming rows on either side of the midrib, cylindrical, 0.2 to 0.4 mm. across and 1.5 to 2.6 mm. high; peridium colorless, rather delicate, rupturing at apex with fringed margins; cells overlapping, majority rhomboid, (10) 15.0–26.6 by 32.0–43.7 μ, inner walls coarsely and closely verucose, not striate, 8.3 to 10.0 μ thick including tubercles, slightly thicker at one end of cell, outer wall, 6.5 to 7.5 μ thick, smooth; aeciospores mostly globoid, occasionally broadly ellipsoid (50) 13.3–20 by 18.3–24.1 μ, standard (19 by 22 μ), wall colorless, 2 to 2.5 μ thick, coarsely and closely verucose, contents colorless.

On living needles of *Abies grandis* and *A. lasiocarpa* from early spring to late fall depending upon elevation.

The Overwintering of Rusts

It has long been a puzzle as to why the Pucciniastrum occurring on species of Epilobium other than *E. angustifolium* (L.) Scop, has not been found to have its alternate stage upon species of Abies. Successful inoculations of Abies sp. with teliosporic material of *P. pustulatum* on *E. angustifolium* have been made in Europe and in America. Fraser[10] secured results in 1912 upon *Abies balsamea* and has collected the aecia in the field. Check results have been secured by the writers[11] in 1916. All experiments were properly controlled.

The uredinial stage of a Pucciniastrum has often been collected in the northwestern states upon *E. adenocaulon* Haus. but never the telial stage. Examination of available exsiccati material fails to disclose any stage of *P. pustulatum* upon *E. adenocaulon* other than the uredinial stage (N. A. U. Nos. 77 and 1087, Fungi Col. Nos. 2575, 2782, 3180, 3773, and 4334, Fungi Dakotensis No. 371, and Jackson's Col. No. 1488). Many collections of the II stage have been made by the writers in months of the year which appear very much out of season for this stage of the rust as the following dates show: March 20, 1916, April 12, 1916, May 4, 1916, June 3 and 15, 1916, July 9, and 14, 1915, August 4 and 20, 1915, September 2, 10, and 28, 1916, October 11, 21, and 28, 1916, November 14, 1916. This indicates a continuation of the uredinial stage throughout the entire year. Examination of all local and acquired collections fails to show where a single collection of the telial stage has been made. On October 18, 1916, three rosettes of *Epilobium adenocaulon* were secured in the field and potted in the greenhouse. Two of the rosettes bore the uredinial stage of *Pucciniastrum pustulatum* on such portions of the leaves as were protected by the outer rosette leaves. All the leaves of the infected rosettes were cut off, care being taken to remove all rusted areas and to cut back the leaves as close to their bases as possible. The only two sources of infection remaining open to the oncoming leaves were the very few urediniospores and the possible mycelium in the portions of the leaves left on the plants. New leaves gradually appeared and on November 1 several of them bore the uredinial stage of the rust. A large number of spores were liberated by these few infections. Germination tests of the spores showed a large percent germinating. A few days later spores were collected which had germinated in situ on the rosettes and produced a small mat of mycelium. Examination of the portions of leaves left on the rosettes after cutting off the infected leaves showed that considerable mycelium was present in the cells of the mesophyll just beneath the epidermis. From November 15 to 22 the rosettes developed a few leaves from two to two and one half inches from the ground, indicating a departure from the strictly rosette habit due to the temperature of the greenhouse. The lowermost ones developed uredinia in abundance. The uppermost leaves as yet showing no infection were sprinkled with urediniospores taken from the pustules beneath on November 18, 1916. On November 27 uredinia developed on the leaves thus inoculated. Two control plants remained normal. The preceding data indicate the presence of a biological species of *P. pustulatum* occurring on *E. adenocaulon* and overwintering by means of mycelium and uredinia upon the rosettes which continue living until spring. The rust is carried over principally by means of urediniospores which reinfect the leaves of the rosette and continue throughout the year infecting the leaves of the flowering stalk during the spring and summer. The fact that this form of the rust on *E. adenocaulon* produces no telia is evidence of its continuation in the uredinial stage and also explains the absence of a corresponding aecial stage upon Abies.

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[10] Fraser, W. P. Cultures of Heteroecious Rusts. Mycologia **4**: 175. 1912.

[11] Weir, J. R., and Hubert, E. E. A Successful Inoculation of *Abies lasiocarpa* with *Pucciniastrum pustulatum*. Phytopathology **6**: 373. 1916.

P. pustulatum occurring upon *E. angustifolium* produces telia which are capable of infecting species of Abies. This plant develops from perennial horizontal root stalks. Rosettes which overwinter are not produced and no evidence has been found to indicate any stage of the rust overwintering on the living plant.

Studies have also been made upon *Coleosporium solidaginis* (Schw.) Thum. occurring upon species of Aster and Solidago.

On October 18 four pots containing rosettes of Aster spp., 2 of *Aster conspicuus* Lindl. and 2 of *A. laevis-gayeri* Grey, infected with the uredinial stage of *C. solidaginis*, were placed in the greenhouse at Missoula, Mont. All of the leaves of the rosettes were removed and the chances for infection depended entirely upon such few urediniospores as had become transferred from the infected leaves. The rust had been mature for some time previous to placing in the greenhouse. On October 28, uredinia appeared on such leaves or portions of leaves as were then present. From this date on other leaves as they appeared became infected and developed scattered groups of uredinia. Four control plants remained normal. Collections of this stage of the rust upon species of Aster and Solidago have been made during the months of the year when only the rosettes of the plants were in evidence. Most of these collections were made in late winter or in early spring before the snow had left the ground. Mains[12] in his article on the overwintering of *Coleosporium solidaginis* produces very good evidence of the overwintering habit of this rust on rosettes of Solidago sp. The collections in Idaho and Montana of infected rosettes of *Solidago missouriensis* Nutt. and *S. canadensis* L. during the months of March and April before the peridia of the aecial stage on *Pinus contorta* have appeared confirms the conclusions of Mains as to the wintering habit of this fungus.

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[12] Mains, E. B. The Wintering of *Coleosporium solidaginis*. Phytopathology **6**: 371. 1916.

The overwintering of a fungus such as *C. solidaginis* on Aster and Solidago spp. when developing in regions so far removed from the alternate host Pinus as to be too remote for infection by spores carried by the wind is a question which has remained unanswered for some time. Clinton[13] refers to this problem in 1907 and comes to the conclusion that the rust winters over in the rosettes principally by means of the urediniospores. A more recent article by Ludwig[14] gives some very substantial evidence leading to his belief that the uredinial stage of *C. solidaginis* on Aster, Solidago, and other hosts propagates itself through the winter upon the rosettes principally by means of urediniospores. He concludes that the evidence is in favor of the rusts being able to maintain a high degree of vigor for a long period without sexual reproduction.

OFFICE OF INVESTIGATIONS IN FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
MISSOULA, MONT.

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[13] Clinton, G. P. Heteroecious Rusts of Connecticut Having a Peridermium for Their Aecial Stage. Report of the Station Botanist **1907**: 369.

[14] Ludwig, C. A. Continuous Rust Propagation without Sexual Reproduction. Proc. Indiana Acad. Sci. **1914**: 219.

ENDOTHIA PIGMENTS. I[1]

Lon A. Hawkins and Neil E. Stevens

The genus Endothia is characterized by a yellow or orange stroma and all known species produce a yellow or buff color in the mycelium and upper layers of the substratum when grown on starchy culture media. In connection with cultural studies of this genus Shear and Stevens[2] first called attention to the fact that certain species when grown on cornmeal or other starchy media produced a bright color, "perilla purple," while the others produce no such color.

Continued study enabled them to divide the genus on the basis of this color production. This division does not, however, coincide with the classification based on morphology. On the basis of spore form the genus is arranged as follows:[3]

Section 1.—Ascospores short—cylindrical to allantoid, continuous or pseudo-septate.

- *E. gyrosa* (Schw.) Fr.
- *E. singularis* (Syd. & Syd.) S. & S.

Section 2.—Ascospores oblong—fusiform to oblong-ellipsoid, uniseptate when mature.

- *E. fluens* (Sow.) S. & S.
- *E. fluens mississippiensis* S. & S.
- *E. longirostris* Earle.
- *E. tropicalis* S. & S.
- *E. parasitica* (Murr.) And. & And.

Of these species the first three uniformly produce perilla purple on such media as cornmeal, oatmeal or rice flasks while the others have consistently failed to produce this color. It is noteworthy that *E. fluens* is included in the group which produces the purple color while *E. parasitica* is not. These two species are so similar morphologically that at one time leading mycologists considered them identical. They are found within the same areas, the United States and Japan, and on the same hosts, Castanea sp. Yet *E. fluens* is a saprophyte while *E. parasitica* is one of the most uniformly destructive fungous parasites known. This is perhaps the only case yet recorded of two closely related fungi, growing on the same host, one of which is a virulent parasite and the other a saprophyte. The two species grow readily and can be easily distinguished on artificial culture media. It is obvious that cultural or physiological differences between *E. fluens* and *E. parasitica* are of great interest.

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[1] Published by permission of the Secretary of Agriculture.

[2] Shear, C. L., and Stevens, Neil E. Cultural characters of the chestnut-blight fungus and its near relatives. U. S. Dept. Agr. Bur. Pl. Ind. Circ. 131: 3–18, 1913.

[3] Shear, C. L., Stevens, Neil E., and Tiller, Ruby J. *Endothia parasitica* and related species. U. S. Dept Agr. Bull. 380. 1917.

It was to study the production of the various colors in species of the genus that the work described in the present paper was taken up. Some attention has been paid to the coloring matter produced by *E. parasitica.* Pantanelli[4] considers the pigment to be a lipochrome but records no experimental work in proof of this statement. Anderson[5] disagrees with Pantanelli on this point. He considers the pigment to be an aurine and quotes unpublished work by Mr. C. T. Thomas to substantiate his view. It was hoped in the present investigation to obtain more evidence on this disputed point.

In taking up the study of the pigments various solvents were tried to see which was most favorable for the extraction of the pigment from the mycelium and the mass of rice upon which the fungi were grown. It was found that the coloring matter of all the species was soluble in ethyl alcohol, and a considerable portion of it readily soluble in ether. Accordingly extracts were made of the culture media and mycelium, with alcohol, at room temperature. The alcohol was evaporated and the residue extracted with ether. The ether extract was then filtered, the ether distilled and the pigments taken up in alcohol again. All tests were made in alcoholic solution unless otherwise noted. The coloring matter was found to be yellow when acidified with either hydrochloric, sulphuric, nitric, phosphoric, or acetic acids. When the acid solution was treated with dilute alkali, sodium, potassium or ammonium hydroxides, or sodium or potassium carbonates, it became a deep red. Apparently all the fungi elaborated pigments which were bright yellow when acidified and red when made alkaline. While the alcoholic extracts from all the fungi reacted in the same general way, there were various nuances of red in the extracts from the different fungi.

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[4] Pantanelli, E. Sul parassitismo di Diaporthe parasitica Murr. per il Castagno. Rendiconti della R. Accademia dei Lincei, Classe di Scienze, Fisiche, Matematiche e Naturali. V. **20**: 366–372, 1911.

[5] Anderson, P. J. The morphology and life history of the chestnut-blight fungus. Bull. Penn. Chestnut Tree Blight Comm. **7**: 1–43, 1913.

A study of the alcoholic extracts from pure cultures on rice[6] of *E. parasitica*, *E. fluens*, *E. fluens mississippiensis*, *E. tropicalis*, *E. gyrosa*, *E. singularis*, and *E. longirostris* was made with a spectrophotometer. With this apparatus measurements were made from which the percentage of light of various wave-lengths transmitted by the solution was calculated. These data were used in plotting the curves of spectral transmission.[7] The alcoholic extracts of stromata of *E. singularis* from chaparral oak, *E. gyrosa*, from beech, and *E. parasitica* from chestnut were prepared by separating the stromata

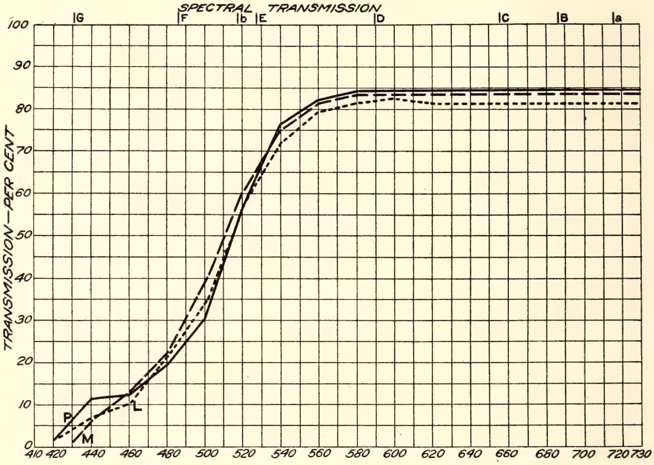

FIG. 1. Curves of percentage of spectral transmission of acidified alcoholic extracts from pure cultures on rice of *E. parasitica* (P), *E. longirostris* (L), and *E. fluens mississippiensis* (M). The curves were plotted with the percentage of light transmitted as ordinates and wave-lengths of light in $\mu\mu$ as abscissae.

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[6] Throughout this study the fungi were grown on rice flasks prepared according to the method published by Shear and Stevens. Loc. cit., p. 13.

[7] This part of the work was made possible through the kindness of Mr. C. G. Peters, of the Bureau of Standards, who made the measurements and calculations.

from the bark and extracting the mass with alcohol. The curves of spectral transmission of these extracts are included in figures 3 and 5.

From the curves shown in figures 1 to 3 it is noticeable that the transmission spectra of the acidified alcoholic extracts of pure cultures of the species of Endothia studied group themselves into three classes. The first of these includes *E. parasitica*, *E. longirostris*, and *E. fluens mississippiensis*. The curves for these three fungi agree rather closely in most cases, the region of greatest variation being in the shorter wave lengths transmitted. The distinctive feature of these curves is that they indicate that practically all the violet rays are absorbed. Only a small portion of the blue is transmitted while most of the yellow, orange and red rays pass through.

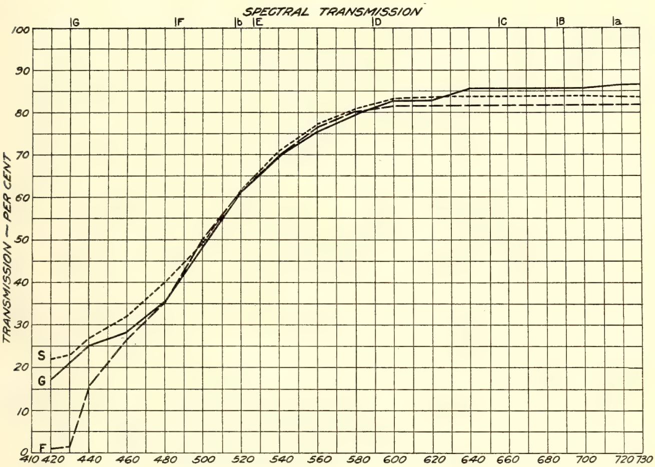

FIG. 2. Curves of percentage of spectral transmission of acidified alcoholic extracts from pure cultures on rice of *E. fluens* (F), *E. gyrosa* (G), and *E. singularis* (S). The curves were plotted with the percentage of light transmitted as ordinates and the wave-lengths of light in μμ as abscissae.

The curves of spectral-transmission for *E. fluens*, *E. singularis*, and *E. gyrosa* make up the second group and are shown in figure 2. An inspection shows that the greatest variation in these curves is again in the shorter wave-lengths. There is some transmission of the violet rays, more of the blue, and a gradual increase in the percentage transmitted through the green and yellow to the orange. From this region through the red the percentage of transmission is practically the same for all wave lengths. The curves of this type are different from those of group I in that more of the violet and blue are transmitted and somewhat less of the yellow.

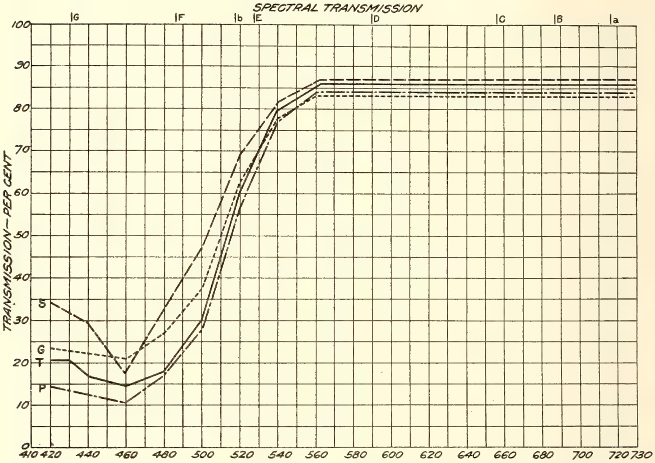

FIG. 3. Curves of percentage of spectral transmission of acidified alcoholic extracts of *E. tropicalis* (T) from pure culture on rice, *E. parasitica* (P) from stromata from chestnut, *E. gyrosa* (G), from stromata from beech, and *E. singularis* (S) from stromata from chaparral oak. The curves were plotted with the percentage of transmission of light as ordinates and the wave-lengths of light in $\mu\mu$ as abscissae.

Figure 3 shows the curves derived from the percentage of light of the different wave-lengths transmitted by the alcholic extract of *E. tropicalis* in pure culture and also the curves for the alcoholic extracts of the stromata of *E. singularis* grown on oak, the stromata of *E. gyrosa* grown on beech and the stromata of *E. parasitica* grown on chestnut. The curve obtained for the extract from *E. tropicalis* is typical of the group. This fungus was grown on artificial culture media and thus is the only one in this group directly comparable with those of the other two groups.

The curves of this group are different from those of type I in that more of the violet and blue are transmitted. They are separated from those of group II by the fact that their minimum percentage of transmission is in the blue. A higher percentage of wave-lengths shorter than 460 $\mu\mu$ and a slightly higher percentage of the yellow is transmitted than in any of the others. The chief difference in these three groups is in the degree of absorption of the shorter wave-lengths of light by the solutions.

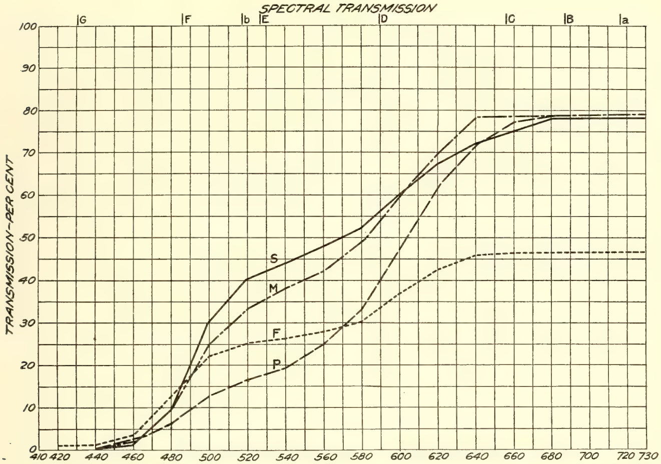

FIG. 4. Curves of percentage of spectral transmission of the alkaline alcoholic extracts of pure cultures on rice of *E. parasitica* (P), *E. fluens*, (F), *E. fluens mississippisensis* (M) and *E. singularis* (S). The curves were plotted with the percentage of light transmitted as ordinates and wave-lengths in $\mu\mu$ as abscissae.

The curves derived from the percentages of spectral transmission of the alcoholic extracts were considerably different when these solutions were made alkaline. They group themselves into two classes and the curves within each class are more widely divergent than in the acidified solutions. As in the acidified extracts one striking difference between these two groups is in the transmission of the shorter wave-lengths. In figure 4, which shows the curves of spectral transmission of *E. parasitica*, *E. fluens*, *E. fluens mississippiensis*, and *E. singularis* grown in pure culture, it is apparent that very little of the light of a wave-length below 480 $\mu\mu$ is transmitted. From this point the percentages of transmission increase with increase in wave-length.

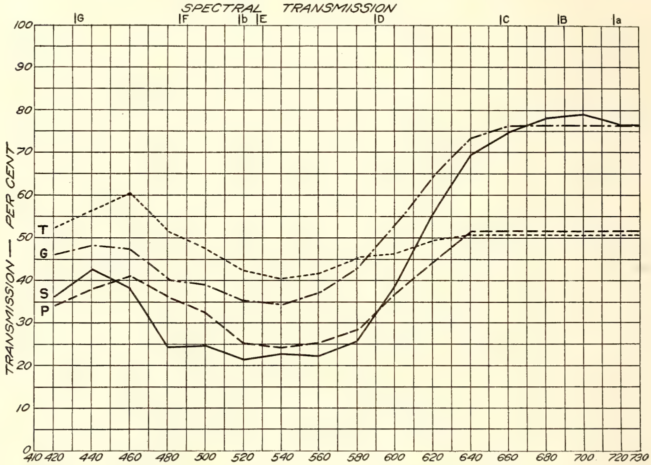

FIG. 5. Curves of percentage of spectral transmission of alkaline alcoholic extracts of *E. tropicalis* (T) from pure cultures on rice, *E. parasitica* (P), stromata from chestnut, *E. gyrosa* (G), stromata from beech, and *E. singularis* (S) stromata from chaparral oak. The curves were plotted with the percentage of light transmitted as ordinates and wave-lengths of light in $\mu\mu$ as abscissae.

The curve of the percentages of transmission of the alkaline extract from the mycelium of *E. tropicalis* in pure culture shown in figure 5 is an example of the other type of curve. With this solution about 52 percent of the light of a wave-length of 420 $\mu\mu$ passed through and 60 percent of the wave length of 460 $\mu\mu$. This is the maximum transmission of this solution.

The other three curves shown in figure 5 were derived from the percentages of light transmitted by the alkaline alcoholic extracts from

E. gyrosa, E. singularis, and *E. parasitica,* stromata on beech, chaparral oak, and chestnut respectively. These curves are the same type as that obtained for *E. tropicalis,* but in the case of the extracts from *E. gyrosa* and *E. singularis* considerably more light was transmitted in the longer wave-lengths.

The chief difference between these two groups, as shown in figures 4 and 5 is in the percentage of violet and blue light transmitted by the extracts.

In the foregoing pages it has been shown that there were three types of curves of the percentage of spectral transmission in the acidified alcoholic extracts of these seven species of fungi. This fact seemed to indicate the presence of several different pigments in the cultures. The differences in spectral transmission might of course be due either to the presence of different pigments, or to the presence of the same pigments in different proportions. Investigations were accordingly carried out to see if there was any common pigment and to determine if possible the presence of other pigments. As it was hardly possible to investigate carefully the pigments formed by all the species, forms typical of the three groups were chosen for study. Those selected were *E. parasitica* as a type of group I, *E. fluens* from group II and *E. tropicalis* from group III.

The fungi were grown on rice in Erlenmeyer flasks until the medium was well covered with mycelium and showed a considerable amount of color. This usually required from three to six weeks. The culture medium and mycelium were then removed from the flasks and dried at a temperature of about 60° C. The dried mass was ground, placed in a percolating funnel and extracted with cold neutral alcohol. The extract thus obtained was concentrated under reduced pressure until nearly all the alcohol was driven off. The residue was washed into a precipitation jar with water and from 10 to 15 volumes of water added. A reddish yellow precipitate resulted. This precipitate was collected on a filter and extracted with ether until the solvent came through nearly colorless. It was noticeable that a residue always remained on the filter and was especially large in the preparation from *E. fluens.* The ether extract was concentrated and four volumes of petroleum ether added to it. A yellow amorphous precipitate of pigment settled out. This pigment was separated by filtration, dried, redissolved in ether and precipitated out with petroleum ether. Pigments were obtained from all three fungi which, judged by their appearance, their solubility in ether and alcohol and the fact that they were practically insoluble in water, were very similar. No crystalline compound was obtained by this method.

An acetyl derivative was prepared from this yellow pigment by dissolving a quantity in acetic anhydride to which some anhydrous sodium acetate had been added and boiling under a reflux condenser for two and one half hours. It was allowed to cool and was poured into a beaker of cold water and allowed to stand with frequent stirrings for ten or twelve hours. The precipitate which had formed was separated from the solution, dissolved in absolute alcohol and cleared with animal charcoal. It crystallized from the alcoholic solution in yellow needles. After recrystallizing three times the melting point was determined and found to be between 240° and 243° C. uncorrected. The acetyl derivatives were prepared from the yellow pigment from all three fungi and were apparently identical. They had the same appearance, solubility, and melting point. There was, however, a considerable difference in the yield of acetyl derivative from the different pigments. The largest yield in proportion to the quantity of pigment used was obtained from the pigment from *E. tropicalis;* the smallest was from the yellow precipitate from *E. parasitica.*

The acetyl derivative was broken down and the original pigment recovered by dissolving the crystals in concentrated acetic acid, with heat, and then adding a drop or so of concentrated sulphuric acid and warming again. Several volumes of water were then added and the pigment was precipitated out. The mixture was filtered and then washed with water and dissolved in alcohol. The acetyl derivatives of this yellow pigment from all three species of Endothia were broken down in the same way. The three alcoholic solutions were treated with a number of common reagents and reacted in exactly the same way in all cases. It is evident that the three species of Endothia produce the same pigment when grown on rice flasks and, as these three species are typical of the three groups mentioned earlier in this paper, it is highly probable that all the species of Endothia studied produce this pigment. This pigment will be designated pigment *A* throughout the rest of this paper. It is soluble in acetic acid, ether, carbon-tetrachloride, and a number of other organic solvents. It is slightly soluble in petroleum ether. It dissolves with a green color in sulphuric acid and in concentrated nitric acid. When acidified it is yellow and is insoluble in water. When made alkaline it has a color approaching crimson magenta. The dry pigment is soluble in dilute aqueous solutions of sodium or potassium or ammonium hydroxides, or sodium or potassium carbonates.

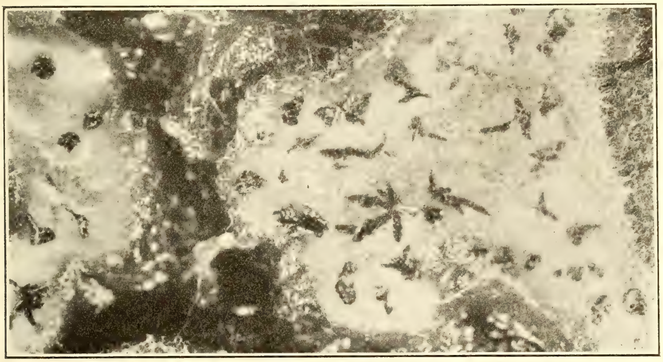

FIG. 6. Photomicrograph of crystals of pigment *B* on the mycelium of *E. fluens* grown on rice. (The writers are indebted to Dr. Erwin F. Smith for this photograph.)

As was mentioned earlier, not all pigments extracted from the ground-up rice and mycelium with alcohol were readily soluble in ether. The residue remaining after the ether extraction in the case of the pigment mixture from *E. fluens* was considerable. The cultures of this fungus on rice, as has been pointed out, show a brilliant purple color in the medium while the mycelium on top is yellow. In the present study a red pigment frequently crystallized out on the mycelium of *E. fluens*, fig. 6. These crystals were not observed in cultures of *E. parasitica* or *E. tropicalis*. It was noticed also that when the concentrated alcoholic extract was treated with water the water was a brilliant red.

These facts seemed to indicate that part of the pigment *A* was alkaline and soluble in water and slightly soluble in ether, or that some other pigment was formed by this species of Endothia along with pigment *A*. The red watery solution obtained from the first precipitation of the yellow pigment was evaporated to dryness and taken up in hot dilute alcohol. The dark red solution thus obtained was concentrated in a desiccator and a red crystalline precipitate was formed. The residue from the first precipitation of the pigment of *E. fluens* after the extraction with ether was treated with alcohol. The greater part of this residue dissolved. When this solution cooled a red crystalline precipitate was formed similar to that obtained from the watery extract mentioned above. The crystals were red glistening plates and were optically active. After two recrystallizations an acetyl derivative was prepared according to the method already described. The compound crystallized out of absolute alcohol in colorless needles. It was recrystallized twice and dried. It melted at 196° to 197° C. uncorrected. A portion of the acetyl derivative was broken down with sulphuric acid after the method followed with the acetyl derivative of pigment *A*, and a pigment was obtained which had much the same appearance as the original red pigment, which will be designated as pigment *B* in this paper.

The properties separating pigment *B* from pigment *A* are: It forms a different acetyl derivative, is only slightly soluble in ether, insoluble in toluene, carbon-tetrachloride, petroleum ether, and concentrated nitric acid. It is soluble in water and may be crystallized from a water or dilute alcohol solution. When a dilute solution is treated with a drop of ferric chloride it becomes darker, assuming a greenish raw-sienna color. The alcoholic solution when made slightly alkaline closely approaches orange vermillion in color. Crystals of the red pigment found on the mycelium of *E. fluens* were removed and tested with various reagents. The reactions were apparently the same as those just described.

Pigment *B* has not been discovered in cultures of either *E. parasitica* or *E. tropicalis* grown on starchy culture media. It may be elaborated by these two fungi, but if so it occurs in such small amounts as to render detection exceedingly difficult.

It was mentioned in the description of the work with pigment *A* that the yield of acetyl derivative obtained from the yellow precipitate from the extract from *E. parasitica* was very small as compared to the yield from a similar amount of the yellow pigment from *E. tropicalis* or *E. fluens*. It was also shown that the alcoholic solution of the pigment from *E. parasitica* grown on rice has a considerably different spectral transmission than that from *E. tropicalis*. These considerations made it seem quite possible that another pigment might be present in the solution in addition to pigment *A*.

The alcoholic solution remaining after the acetyl derivative of the pigment from *E. parasitica* had crystallized out and had been separated was treated with about four volumes of water. A flocculent yellow precipitate was formed. This was filtered off and washed with water. The dry pigment was amorphous and of a bright yellow color. No acetyl derivative was formed even on long boiling with acetic anhydride and sodium acetate. When the pigment was dissolved in alcohol and treated with dilute alkali, the color closely approached rose doré. It is evidently another pigment and the properties which separate it from pigment *A* are as follows: It does not form an acetyl derivative; it has an entirely different color when treated with alkali; it is insoluble in cold petroleum ether and dissolves in cold concentrated nitric acid with an orange red color and is red when dissolved in cold sulphuric acid. It is readily distinguished from pigment *B* by its solubility in ether and nitric acid and insolubility in water and very dilute alcohol, also by its appearance when dry and when in acid or alkaline solution. This pigment, which will be referred to in this paper as pigment *C* is also found in extracts from *E. fluens*. Its presence has not yet been demonstrated in the extract from *E. tropicalis*.

From the experimental work just described it is evident that there are at least three different pigments formed by species of this genus, pigment *A*, apparently common to all species, pigment *B* found in *E. fluens* and probably also in the other species having a similar spectral transmission of the acid alcoholic solutions and pigment *C* which is present in the two groups typified by *E. fluens* and *E. parasitica*.

It is of course quite possible that these three pigments are closely related chemically and may be derivatives of the same substance. They are similar in many particulars. All three are composed of carbon, hydrogen and oxygen. That is, on incineration they leave no ash and tests for nitrogen,[8] phosphorus, sulphur and the halogens showed that none of these elements were present.

The comparative solubilities of these three pigments are shown in Table I. These tests were all at room temperature and indicate whether or not the pigment is appreciably soluble in the reagent used.

From the table it is evident that these pigments, especially pigments *A* and *C*, are readily soluble in a considerable number of organic solvents. They are not in all cases soluble in the same reagent, however, and these points of difference are of use in distinguishing and separating the pigments.

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[8] Fresenius, C. R. Quantitative Chemical Analysis. Cohn's translation of the sixth German edition, 2: 4–7. 1911.

TABLE I

Solubility of Pigments A, B and C, in Various Reagents at Room Temperature

Reagent	Pigment		
	A	B	C
Acetic acid	Soluble	Soluble	Soluble
Acetic ether	“	“	“
Acetone	“	“	“
Ethyl alcohol	“	“	“
Methyl alcohol	“	“	“
Amyl-alcohol (normal)	“	“	“
Ether	“	Slightly soluble	“
Toluene	“	Insoluble	“
Benzol	“	Soluble	“
Carbon-tetrachloride	“	Insoluble	“
Chloroform	“	“	“
Carbon-bisulphid	“	“	“
Petroleum ether	Slightly soluble	“	Insoluble
Sulphuric acid	Soluble	Soluble	Soluble
Nitric acid (conc.)	“	Insoluble	“
Water	Insoluble	Soluble	Insoluble

The statement by Pantanelli that the coloring matter of *E. parasitica* is a lipochrome seems hardly to be corroborated by the evidence brought out in these experiments. Lipochromes according to Zopf[9] and Samuely[10] break down readily when exposed to light and air. They are soluble with a green color in concentrated sulphuric and nitric acids. When saponified by boiling with dilute sodium hydroxide they are insoluble in alcohol. According to Zopf they are soluble in petroleum ether and insoluble in water.

The pigments obtained in this study did not break down when exposed in solution to light and air. Solutions were kept in the laboratory for more than a year without apparent deterioration. Pigment *A* was the only pigment which gave a green color when dissolved in concentrated nitric or sulphuric acids. Pigment *B* was insoluble in this last mentioned reagent. Boiling in a dilute solution of sodium hydroxide did not, apparently, affect the solubility of the pigments in alcohol. Pigment *A* was the only pigment soluble in cold

[9] Zopf, W. Die Pilze. Page 144. Breslau, 1890.

[10] Samuely, F. Abderhalden's Handbuch der Biochem. Arbeitsmethoden II: 758, 1910.

petroleum ether. Pigment B was soluble in water. It is obvious then that these pigments are lacking in many of the properties of lipochrome and there is little reason at present for assuming that they belong in this rather indefinite group.

There is some evidence to support the conclusion of Anderson that the pigment in *E. parasitica* is aurine. According to Rota's[11] system for the classification of coloring matters these two pigments might be classed as aurin.

Aurin is the trade name applied to a red pigment obtained by heating phenol and oxalic acid with sulphuric acid. According to Dale and Schorlemmer,[12] it was applied to this preparation as prepared by Kolbe and Schmitt.[13] Dale and Schorlemmer found this preparation to be a mixture of compounds and succeeded in separating out what they considered pure aurin. The name aurin is retained by Schultz and Julius[14] and by Allen[15] for para-rosolic acid which according to Allen has a formula $C_{19}H_{14}O_3$. This dye, however, is insoluble in benzol and carbon-bisulphide and by boiling with sodium hydroxide and zinc dust it is decorolorized. This is not true of the pigment from *E. parasitica*. Moreover, a comparison of pigments *A* and *C* in both alkaline and acid solutions with solutions of commercial aurin shows that they are not the same color.

Other points of difference might be mentioned. It is, however, apparent that while aurin and the pigments from *E. parasitica* have some properties in common the conclusion that they are the same is unwarranted. Whether any of the three pigments considered in this paper are similar in structure to aurin is a problem which needs further investigation. It lies within the scope of this paper to take up the chemical and physical properties of these pigments only in so far as is necessary for separating and distinguishing them from each other. Their chemistry is under investigation and will be considered in a later paper.

[11] Wiley, H. W., et al. Official Methods of Analysis of the Association of Official Agricultural Chemists. U. S. Dept. Agr. Bur. Chem. Bull. 107. 1910.

[12] Dale, R. S., und Schorlemmer, C. Ueber das Aurin. Ber. Deutsch. Chem. Ges. 4: 574–576. 1871.

[13] Kolbe, H., u. Schmitt, C. Rother Farbstoff aus dem Kreosot. Annalen der Chemie und Pharmacie. **119**: 169–172. 1861.

[14] Schultz, G., u. Julius, P. Tabellarische Übersicht der Künstlichen Organischen Farbstoffe. Dritte Auflage. 124, 1897.

[15] Allen, Alfred, H. Commerical Organic Analyses. ed. 3. 3: 310–311. 1902.

Early in this work it became apparent that all the members of the genus studied elaborated pigments which were bright yellow when acidified and red when alkaline. This suggested the possibility that if *E. parasitica* could be grown on a sufficiently alkaline medium it might produce the purple color considered characteristic of *E. fluens*. The writers were able to suppress the production of the purple color in cultures of *E. fluens* by the addition of 10 cc. n/10 sulphuric acid to each 100 cc. culture flask. Cultures of *E. parasitica* were made to produce a wine color in the culture media by the addition of 2 grams of calcium carbonate to each 100 cc. flask before sterilizing.

While not particularly significant, these tests furnish another example of the necessity of carefully standardizing culture media used in critical comparative study of fungi. This is especially true since no character is more commonly used to distinguish species of fungi in pure culture than the production or nonproduction of color changes in the mycelium or culture media. Under carefully controlled cultural conditions the ability to produce color on certain media may be a distinguishing character of great value, as in the work of Appel and Wollenweber[16], Grossenbacher and Duggar,[17] Thom,[18] and others.

In studying the growth of species of Endothia on liquid media it was noticed that *E. parasitica* produced a red coloration in old cultures when the medium contained peptone. This was found to be due to the ammonia liberated by the growth of the fungus acting on the yellow pigment. Anderson (loc. cit., p. 14) mentions the fact that old cultures of *E. parasitica* often become purple or wine colored and attributes this change to the fact that the fungus in its growth on the agar gradually causes it to become alkaline, thus changing the pigment from yellow to purple. The writer's investigations indicate that these conclusions were probably correct and that the change to an alkaline reaction may have been due to the formation of ammonia in the cultures.

In the experimental work described it has been shown that three different pigments are elaborated by the fungi in this genus. It has been shown further that the curves of the percentage of spectral transmission of the acidified alcoholic extracts of the pigments from the seven different fungi group themselves into three distinct classes. An investigation of the pigments produced by typical fungi from each of these three classes, *i. e.*, *E. parasitica*, *E. fluens*, and *E. tropicalis*, show that there is one pigment common to all three groups but that each of the three fungi is characterized by some one of the three pigments.

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[16] Appel, O., and Wollenweber, H. W. Grundlagen einer Monographie der Gattung Fusarium (Links). Arbeiten aus der Kaiserlichen Biologischen Anstalt für Land- und Forstwirtschaft. **8**: 1–207. 1910.

[17] Grossenbacher, J. G., and Duggar, B. M., A contribution to the Life-history, Parasitism, and Biology of *Botryosphaeria ribis*. N. Y. Geneva Agr. Exp. Sta. Tech. Bull. 18. 1911.

[18] Thom, Charles. The *Pencillium luteum-purpurogenum* group. Mycologia **7**: 134–142. 1915.

E. tropicalis apparently elaborates only pigment *A* in quantity, although a very little of pigment *C* may be present. *E. parasitica*, the type of the group which contains also *E. longirostris* and *E. fluens mississippiensis*, secretes pigment *A* in small amounts, but pigment *B* was not found at all. Pigment *C* is characteristic of this group. The group containing *E. fluens*, *E. gyrosa*, and *E. singularis*, of which *E. fluens* is considered typical, is apparently the only one of the three which secretes all the pigments. Pigment *B* is found only in this group, and is thus characteristic of the group. This pigment is soluble in water and is the cause of the "perilla purple" color in cultures of this fungus. It frequently forms crystals on the mycelium (fig. 6).

It is evident from this work that the curves of percentage of spectral transmission shown in figures 1 to 5 are in most cases curves of mixtures of these pigments, and that the difference in the curves of spectral transmission for the three groups is due to the fact that different pigments predominate in the alcoholic extracts from the fungi of these three groups. It is probable that further investigation with quantitative methods would show that the variation in the curves for different members of the same group was due to the presence in varying proportions of the pigments characteristic of that group.

It is of interest to note that the grouping of the species based on the spectral transmission of the acidified alcoholic extracts of the mycelium shows no apparent agreement with the division based on morphology, host, or geographical distribution. *E. tropicalis*, which apparently produces only pigment *A*, differs to be sure from all the other fungi examined in host and geographical distribution, being known only from Ceylon on Elaeocarpus. It is, however, rather closely related morphologically to *E. parasitica* and other members of this group.

The group characterized by a curve of spectral transmission indicating the presence of pigments A and C contains only forms having oblong fusiform to oblong ellipsoid ascospores, and with somewhat similar stromatic characters. The members differ widely, however, in host and geographic relations, *E. longirostris* being a tropical form known at present only from Porto Rico and French Guiana. *E. parasitica* and *E. fluens misissippiensis* occur on the same hosts, Castanea sp. and Quercus sp., but *E. parasitica* is the destructive chestnut blight organism known already from China, Japan, and the United States, while *E. fluens mississippiensis* is a weak saprophyte which has been found in only four localities in the United States.

The group characterized by a spectral transmission curve indicating the presence of all three pigments contains species widely different in morphology and distribution. *E. gyrosa* and *E. singularis* have cylindrical ascospores and are found only in the United States, *E. singularis* only on the chaparral forming species of Quercus in Colorado and New Mexico. *E. gyrosa* is found on species of Quercus, Fagus, Castanea, and Liquidambar, and is widely distributed in this country, though abundant only in the southeastern portion. *E. fluens*, on the other hand, is a cosmopolitan species found in the United States on Castanea and on Quercus in Europe and Asia on a variety of hosts. In their stromatic characters also these species are widely different. The stromata of *E. singularis* are large and irregular, being 3–5 mm. wide by 2–4 mm. high, and disintegrate into a powdery mass when the wall is ruptured. The stromata of *E. fluens* are much smaller, being only .75–3 mm. in diameter by .5 to 2.5 mm. high and very compact.

On the other hand, the two most closely related fungi of the genus, *E. fluens* and *E. fluens mississippiensis*, fall in different color groups. In fact, it may well be that the production of pigment B by *E. fluens* is the chief character which distinguishes it from its variety. The varietal name was proposed by Shear and Stevens to designate a form which they were unable to separate from *E. fluens* on morphological grounds, but which showed constant differences on culture media.

The fact that the red pigment in *E. fluens* is not found in *E. parasitica* grown on the same media and under the same conditions is of especial interest since the two species are so much alike morphologically and grow on the same host, yet differ so widely in their relation to their hosts, *E. parasitica* being, as has already been pointed out, the uniformly destructive chestnut blight parasite, while *E. fluens* is a harmless saprophyte.

The fact deserves more than passing notice also that what is apparently the same pigment is produced by all the known members of a genus, which, although small, includes species from four continents, from both temperate and tropical climates and occurring on rather unrelated hosts.

Bureau of Plant Industry,
Washington, D. C.

OBSERVATIONS ON AN ACHLYA LACKING SEXUAL REPRODUCTION

Wm. H. Weston

The following paper embodies the results of the writer's investigation of a Saprolegniaceous fungus which shows the zoospore production characteristic of the genus Achlya, but which lacks sexual reproduction. The resistant function exercised in most species of Achlya by the sexually produced spores is apparently assumed, in the form to be described, by large, heavy-walled spores of non-sexual origin, and sharply defined morphological characteristics. Structures of a similar nature have been recorded in other Saprolegniaceae; but this form is unique in that its reproduction is limited to the zoosporangia and to these structures which are of a particularly distinct type. In contradistinction to the zoospores, these bodies are resistant to unfavorable conditions. It seems advisable, therefore, to avoid the implications of the names "conidia," "chlamydospores," "gemmae," "Sporangienanlage," "resting sporangia," or "resting spores," generally used for these structures; and to employ simply the term "resistant spores."

The writer realizes only too well the incompleteness of this study; and regrets that the untimely destruction of stock cultures has prevented the further physiological and cytological investigations which had been planned. These results are presented for publication in the hope that they may prove of interest to those working in the same field.

MATERIAL AND METHODS

The fungus appeared on dead flies dropped into a culture containing sediment and algae from a stone watering trough at Waverly, Mass. For over two years gross cultures of the fungus were successfully maintained in battery jars cooled in running water, and covered with glass to exclude the dust.

Pure cultures were obtained by the following methods.

1. A young sporangium was washed repeatedly in sterile water, and allowed to discharge zoospores in a drop of sterile water on a

slide. This drop was then added to 2 or 3 cc. of sterile water in an atomizer, and the resulting mixture sprayed on the surface of 2 percent beef-extract agar in petri dishes. By examination with a binocular microscope, the positions of single isolated zoospores were noted and marked. After two or three days of growth in a cool place, bits of the uncontaminated peripheral portions of the mycelia arising from these zoospores were transferred on agar chips to fresh nutrient substratum.

2. A large number of vigorous resistant spores were washed repeatedly in sterile water and plated out in a separation culture series of four or five plates of 2 percent beef extract agar. The spores were sufficiently resistant to remain uninjured by their exposure to the hot agar, and promptly germinated. In the last two or three plates some of the mycelia arising by germination of the isolated resistant spores were found to be uncontaminated; and from them transfers were made to fresh nutrient material.

When uncontaminated mycelia of the fungus had been obtained by the above methods, stock cultures were maintained on firm cornmeal mush in 500 cc. flasks. For the investigation of the fungus, mycelia were grown in petri dishes in nutrient solutions of beef-extract, and of pea, corn, bean, and other vegetable decoctions, and transferred to sterile water or to various solutions in petri dishes or hanging drop cultures for further development according to the methods already worked out by Klebs (7), Kauffman (6), Obel (13), Pieters (14), and others.

DEVELOPMENT OF THE FUNGUS

The normal life cycle of the fungus comprises the establishing of a branched mycelium which gives rise to large numbers of zoosporangia of the Achlya type. At first these zoosporangia are produced exclusively; but gradually they are superseded by abundant resistant spores which continue to be formed until the mycelium is exhausted. This regular cycle was observed for a space of two years in the original gross culture in which the fungus appeared; and since the conditions there probably closely approximated those of its natural habitat, we may infer that it would follow the same cycle in nature.

In battery-jar cultures like those mentioned above, species of several Saprolegniaceous genera have been found by the writer to maintain their normal cycles of sexual as well as non-sexual reproduction for long periods of time. This Achlya, however, under these conditions continued for two years to follow its cycle of unbroken non-sexual reproduction. Under pure culture conditions, also, mycelia, grown on sterilized flies, or transferred from various nutrient solutions to water, developed Achlya zoosporangia followed only by resistant spores.

The reactions of the fungus when subjected to culture conditions designed to induce sexual reproduction will be discussed later, the phases of its normal development now being considered in detail.

Zoosporangia.—The vegetative mycelium of the fungus, whether in gross or pure culture, consists of non-septate, branching hyphae that show no apparent difference from those of other species of Achlya. In gross cultures, as has been stated above, the first reproductive structures to which the mycelium normally gives rise are zoosporangia. In pure cultures, well nourished mycelia can be induced to form zoosporangia in abundance by a distinct and rapid decrease of the food supply according to the methods of Klebs (7), Kauffman (6), Horn (4), and Pieters (15).

The process of sporangium development and spore formation in this Achlya is quite normal, agreeing in its external features with the description of Ward (17) for *Achlya polyandra* de B., and Humphrey (5) for *Achlya Americana* Humph. No detailed description need therefore, be given here.

The fully developed sporangia are cylindrical (Fig. 12) to fusiform (Fig. 1) in shape, and vary greatly in size. They are formed in basipetal series, or renewed by side branching (Fig. 12), but never by growth of the sporangiophore through the empty sporangium.

Escape of the sporangiospores from the sporangium is through a terminal papilla of dehiscence; and in connection with the mouth of the sporangium is formed the sphere of the encysted spores (Fig. 1) which is characteristic of the genus Achlya. From each encysted spore (Fig. 2) thus situated there may emerge under the proper conditions a zoospore of the laterally biciliate Achlya type. The encysted spores average 10.5 μ in diameter while the zoospores measure about 12.5 μ by 9 μ.

In shape these zoospores are ovoid with a flattened side bearing a longitudinal groove or sinus from which arise the two cilia (Fig. 3). It is unfortunate that investigators have been content to describe and figure this type of zoospore in the Saprolegniaceae as "bean" or "kidney" shaped. A careful examination of the living spores under a Zeiss J water-immersion lens readily proves these terms to be inadequately descriptive or even misleading. Edson (2) for his most interesting new genus Rheosporangium describes a similar type of spore (p. 285) as "plano-convex or slightly concavo-convex, with a central vacuole, and on the flattened side a sinus from the bottom of which the two cilia of unequal length arise." The description and the accompanying figures agree in general with the writer's observation of this type of spore in Achlya, Thraustotheca, Dictyuchus, and other genera of the Saprolegniaceae.

Upon emerging from the cysts the zoospores swim actively about for as long as thirty minutes if in pure water, and then round off, lose their cilia, and encyst (Fig. 4). These encysted zoospores germinate either directly or after a period of rest according as available nutriment is present or absent. Germination takes place by the outgrowth of a tube (Fig. 5) which shows scanty development, or which ultimately forms an extensive mycelium, the amount of growth being proportionate to the amount of available nutriment.

Resistant Spores.—In the details of its life history as considered thus far, the fungus is a typical Achlya. The formation of a hollow sphere by the sporangiospores at liberation, the character of the zoospores which emerge, and the method of sporangium renewal are distinguishing characteristics of the genus. In its further development, however, the fungus differs from any Achlya hitherto recorded in that zoosporangium formation is normally followed by the production not of sexual organs, but of heavy-walled resistant spores of a very distinct type. Both in gross and in pure culture, formation of these resistant spores follows the production of zoosporangia with remarkable regularity. Resistant spores are produced exclusively, however, under the conditions found by Klebs (7) to inhibit the formation of zoosporangia, although favoring the development of "gemmae." Particularly is this true of 1 percent agar solution.

Although this Achlya is truly an aquatic fungus, these resistant spores are produced in culture not only in liquids but also aerially on solid media. In flask cultures on fairly dry cornmeal they are formed in especial abundance. When such a culture is inoculated with a bit of mycelium, a vigorous growth ensues, covering the surface of the medium from which innumerable stout hyphae with dense whitish content push up into the air. On examining such a culture with a binocular microscope after three or four weeks, large numbers of resistant spores can be seen especially around the edge of the culture.

Most of these spores are formed in chains, and in some cases the terminal spores abjuncted by the pressure of the spores below are tipped over sideways, although still remaining attached. That these resistant spores should be borne aerially is of interest because of the close analogy presented to the aerially borne conidia of certain of the Peronosporaceae. It does not seem justifiable, however, to regard this phenomenon as of any further significance than an instance of convergenec, of parallel development, in the two families.

The process of resistant spore formation takes place as follows: The tip of a hypha becomes densely filled with coarsely granular protoplasm carried up by the streaming of the currents in the peripheral protoplasm. Gradually more and more material is accumulated in the tip forming a dense mass which slowly extends downwards to the base of the spore initial. Meanwhile the tip of the hypha swells to the spherical or oval shape of the mature resistant spore; and finally the spore initial is separated from the hypha by a wall. Around the inner surface of the terminal cell thus cut off, a wall of varying thickness is laid down by the dense, coarsely granular protoplasm which occupies a peripheral position around a large vacuole (Fig. 9).

After the terminal resistant spore has been formed, as just described, other resistant spores are generally formed in basipetal succession under the first as in figures 6, 9, and 10, giving a torulose series closely resembling the catenate oogonia of *Saprolegnia torulosa* de B. In gross cultures, the resistant spores are generally formed in this fashion, giving a very striking and characteristic appearance to the plant. After a time the fragile outer wall of the resistant spore is ruptured (Fig. 9), and the spore is set free in the water to be washed about and then to slowly settle to the bottom. Further development varies with the conditions of the environment, and will be considered later.

The resistant spores are generally formed in a terminal series as above described, but they may be intercalary (Figs. 7 and 8) or may form branching systems of various types. In shape the resistant spores are spherical, cylindric, oval, or less often pyriform, or clavate. Under conditions closely approximating those of nature, the resistant spores are spherical (Fig. 10) or oval; and on fairly dry cornmeal cultures, they are the same. On vegetative mycelia transferred to sterile water and to solutions of various sorts, the resistant spores which are formed are at times cylindrical or club-shaped (Figs. 1 and 12). In solutions of harmful concentration or at a temperature approaching the maximum, they tend to be irregular in shape (Fig. 8). In size the spores average about 110 μ in diameter, the size being apparently correlated with the vigor of the mycelium. The walls of the resistant spores formed aerially on rather dry cornmeal, or in water cultures kept cold, are thick (Fig. 10); but in spores grown in various solutions at laboratory temperature, the thickness is often less (Fig. 12). By chloroiodide of zinc the walls are colored not blue, but a muddy brick-red, showing that their composition is not of pure cellulose, and recalling the reaction of the oogonial walls of other Saprolegniaceous species.

Germination of the resistant spores varies with the environmental conditions to which these bodies are subjected. In water in the absence of food material, a tube is sent out which, after greater or less growth, gives rise to a perfectly normal Achlya sporangium (Fig. 12). In nutrient solutions, however, or on nutrient agar, germination is invariably siphonoblastic, the tube or tubes of germination rapidly giving rise to an extensive mycelium (Fig. 11). At laboratory temperature the resistant spores germinate in from twenty-four to forty-eight hours in the presence of nutriment, and somewhat more slowly in pure water. Germination need not be preceded by a period of rest; since no particular difference in behavior was observed between spores just formed and those two to four weeks old. The spores are very resistant to cold, those from one culture surviving an exposure to outdoor conditions during two winter months in which the water was repeatedly frozen and thawed. The writer has made no extensive investigation of the degree of resistance to extremes of temperature or to desiccation shown by resistant spores. There has been demonstrated, however, a degree of resistance that may perhaps be regarded as an indication that these spores play for the fungus the resistant rôle usually assumed in others of the family by spores of sexual origin.

The formation of zoosporangia and resistant spores as described above completes the reproduction of the fungus. Under natural conditions of growth, the formation of sexual organs was never observed to take place throughout the two years in which the fungus was under investigation. Many attempts to induce the formation of oogonia and antheridia were made without success. Hoping to stimulate sexual reproduction vigorous mycelia were subjected to the influence of the solutions by means of which Klebs (7), Kauffman (6), Obel (13), and others, had successfully induced the formation of sexual organs in other members of the family. Various concentrations of haemoglobin, of leucin, of potassium, sodium, and calcium phosphates, and of other substances were tested; but in no case was the production of sexual organs achieved. The effect of various temperatures and of changes in temperature was also tried; and the rapid or gradual drying of cultures was attempted. Moreover starving was resorted to, the fungus being grown on synthetic media containing a minimum of nutriment or on such natural substrata as pomace flies that had been thoroughly leached out and then dried. Dwarfed plants were produced, but no oogonia or antheridia developed. In all the cases above, a more or less copious formation of resistant spores resulted; but no production of sexual organs occurred.

DISCUSSION

The formation of bodies of non-sexual origin resembling to some extent the resistant spores of the foregoing description has been recorded in nearly every genus of the Saprolegniaceae. In most of the cases on record these structures appear to be transient resistant stages which do not arise under conditions of the environment favorable to zoospore formation, but are induced by extremes of temperature, by foul water, or by other unfavorable conditions. As Klebs (7) has shown, the production of "gemmae" (as he termed these structures) takes place when the fungus mycelium is subjected to conditions of environment permitting of growth and yet prohibiting zoospore formation. The conditions to which the Saprolegniaceae were subjected during investigation, before methods of pure culture were introduced, quite generally resulted in the formation of numerous structures of this sort. No reference to the occasional descriptions of these bodies need be made here, since the literature has been carefully covered in the monographs of Fischer (3) and von Minden (12). It may not be amiss, however, to recall one interesting instance discussed in an early paper by Walz (16). In *Saprolegnia dioica* Prings. he described a type of reproduction which at that time was unknown for the Saprolegniaceae, namely, the formation of the thick-walled bodies produced in basipetal succession at the ends of the hyphae. The resemblance between these structures (cf. Walz, Fig. 20) and the resistant spores of the Achlya described above (Figs. 6 and 10) is remarkably close. It would be of interest to know whether these bodies were formed in addition to the sex organs or took their place. Walz, however, does not mention the zoosporangia or the sex organs; but from his naming the form so definitely, we must infer that he observed the sex organs of the species.

In quite a different category from the occasionally induced structures just considered, must be placed the resistant bodies which are regularly produced in the life cycle of certain Saprolegniaceae forming sex organs only rarely, or even lacking them entirely.

In the genus Saprolegnia there have been reported a number of forms of this kind. Lindstedt (10) described a species of Saprolegnia which produced zoosporangia and zoospores typical of the genus, followed by spherical, pyriform, or more irregular bodies capable of germinating at once by characteristic Saprolegnia zoospores, or of remaining temporarily inactive and later germinating by a tube or by zoospores on the renewal of favorable conditions. Maurizio (11) also recorded three undetermined species of Saprolegnia which, after the formation of the characteristic sporangia, produced not sex organs but the irregular "Sporangienanlagen" which he considered significant from the phylogenetic point of view. In all probability conditions of culture are responsible for the behavior of the fungi in these instances; since the method employed by both Lindstedt and Maurizio are shown by the exact physiological investigations of Klebs (7) to favor "gemmae" formation, and to hinder the development of sex organs. In like manner the failure of Lechmere's (8) more recently described species of Saprolegnia to form oogonia under continued cultivation may be ascribed to his culture methods. Although Lechmere used pure cultures, he employed as a nutrient substratum egg albumin, a substance readily broken down into injurious compounds by the activity of the fungus. Indeed he even mentions (p. 168) changing the water of his cultures every day to keep them fresh. Had his culture conditions been more favorable, it is reasonable to expect in the light of Klebs's investigations that the sporangia would not have presented such confusing abnormalities of development; and the torulose "gemmae" would readily have given place to the normal oogonia of the species (*Saprolegnia torulosa*) which Lechmere later (9) determined this fungus to be. Recently, however, Pieters (15) has subjected certain species of Saprolegnia to exact physiological investigation with interesting results. One of these forms, *Saprolegnia Kauffmaniana*, characteristically produced numerous sporangia and "gemmae"; but formed sexual organs rarely under normal culture conditions, and in haemoglobin solution only at the specific concentration of 0.025 percent. Another form, *Saprolegnia monoica* var. *vexans*, after reproducing only non-sexually during sixteen months of cultivation on various substrata which had induced sex organs in other forms, finally produced an abundance of oogonia and antheridia under the influence of a combination of *M*/200 levulose and *M*/200 leucin. Moreover, in addition to these two species, Pieters mentioned a Saprolegnia (no. 66) forming numerous single spherical "gemmae," and yet, during eighteen months of cultivation, absolutely failing to respond by the formation of sex organs to the usually successful methods of culture.

In the genus Achlya, also, there have been reported somewhat similar forms which show a lack of sex organs coupled with the formation of resistant structures. Under the name of *Achlya oidiifera* Horn (4) described a form which under long continued observation in pure culture, and with the most exact methods of cultivation, showed consistent non-sexual reproduction. None of the conditions by which Horn induced the formation of great numbers of oogonia in *Achlya polyandra* de B. were successful in the case of *Achlya oidiifera*. Instead, there developed oidia-like hyphal segments which formed zoospores at once, or rested even as long as a month and were still capable of further growth. In his study of *Achlya oidiifera*, Horn observed oogonia only once within an ant egg on which he had grown the fungus; and although no antheridia were found on account of the advanced stage of development, the pits on the oogonium wall, and the eccentric zoospores, suggested *Achlya polyandra* de B. or some other member of the *prolifera* group.

More recently Coker (1) made a careful study in pure culture of *Achlya paradoxa*, a species frequently collected by him in North Carolina, and unique in the aberrant behavior of its zoospores. The fungus when isolated from single zoospores produced zoosporangia and "chlamydospores" in abundance. Although the form was for a long time maintained in pure culture under a variety of conditions, sexual organs proved very rare. They were observed but a few times, and their formation could not be induced by a great variety of substrata including nutrient solutions of various kinds containing organic or inorganic salts.

The peculiar Achlya described by the writer in the earlier part of this paper differs from *A. oidiifera* and *A. paradoxa* in the distinct morphological character of its resistant spores and in the absolute lack so far of any sexual reproduction. The destruction of stock cultures has prevented testing the effect of mixtures of leucin and levulose in the proportions found successful by Pieters (15) in inducing oogonium formation in Saprolegnia. It is possible that this Achlya also would yield to this combination or to some other more suited to its own physiologic idiosyncrasies. In view of such a possibility it does not seem advisable to assign a specific rank and name to the Achlya described above. Both physiologically and morphologically this Achlya differs from the other members of the genus; but further investigation might result in inducing the formation of sex organs, and might prove the fungus to be a variety of some already established species.

The significance of these Saprolegniaceous forms which partially or completely lack sexual reproduction is not at once apparent. The few explanatory theories advanced by early investigators were based on the study of material under unfavorable conditions preceding methods of pure culture; and hence may be disregarded. Even the more modern investigators of such forms offer but little explanation of their significance. Maurizio's theory that asexual resistant spores (Sporangienanlage) are reminiscent of primitive, non-specialized structures from which both sporangia and oogonia have been evolved has been largely nullified by Klebs' exact researches on "gemma" formation. On the other hand, in Klebs' interpretation of "gemmae" as "Hemmungsbildungen" induced by conditions unfavorable to other stages of development, we have an explanation undoubtedly correct in most cases, yet hardly applicable to such distinct and consistently formed structures as the resistant spores of the Achlya that is the subject of this paper.

An extremely significant suggestion, however, has been made by Pieters in a recent paper. Pieters' (15, p. 483) suggestion that "the production of sexual organs may depend on some special combination of conditions, differing, doubtless, for each form" is important in emphasizing the physiological difference existing among the species and varieties of the Saprolegniaceae. The members of the family, probably because they are coenocytic in structure, and are completely submerged in the culture media, are very sensitive to the nature of their environment. Morphologic studies reveal no reason why under the same conditions one form should produce oogonia and antheridia;

while another form seemingly identical should just as persistently fail to produce these organs. The physiologic investigations begun by Klebs, and carried on by a number of others, have gradually resulted in a far more enlightening conception of the family. Because of these investigations, we are, in the opinion of the writer, justified in regarding the Saprolegniaceae as a series of forms ranging from those which normally produce sexual organs, through forms which produce sexual organs only under unusual conditions, to forms which have entirely lost the power of sexual reproduction.

On the basis of this ability to form sexual organs the Saprolegniaceae may, for convenience, be divided roughly into three groups. First there are those forms which are strongly sexual producing an abundance of oogonia and antheridia even under adverse circumstances. Here may be grouped *Saprolegnia monoica* de B., *S. diclina* de B., *Achlya polyandra* de B., *A. prolifera* de B., and others. Second, there may be grouped together those forms which possess the power of sexual reproduction, and perhaps even show a sexual stage as a normal phase of their life cycle under unknown conditions of growth in nature, but which under investigation remain imperfect; because the exact conditions favoring sexual reproduction are not supplied. Here belong *Achlya oidiifera* Horn, *A. paradoxa* Coker, *Saprolegnia Kauffmaniana* Pieters, and others. Finally, there are certain forms which have lost the power to produce any sexual organs whatever. The Achlya that is the subject of this paper, the *Saprolegnia no. 66* of Pieters, as well as other forms not as yet recognized as imperfect, should be placed in this category.

The statement that these fungi are absolutely incapable of sexual reproduction cannot be made logically, of course, until they have been grown under every possible condition. Even if these forms should on later investigation be found to possess latent powers of sexual reproduction, it seems probable that the category of non-sexual Saprolegniaceous forms will still persist. It has long been recognized that the male reproductive organs or antheridia are invariably developed in some Saprolegniaceous species, occasionally in others, and never in some. In the genus Saprolegnia particularly, one can trace all gradations from forms normally developing antheridia in abundance, through forms in which antheridia occur rarely but may be induced, to truly parthenogenetic forms. In the opinion of the writer, a quite similar condition also obtains with regard to the oogonia of the Saprolegniaceae.

The lack of sexual reproduction, of a "perfect stage," is not uncommon in the fungi in general. The resemblance of the condition existing in the Achlya which is the subject of this paper to that of certain Fungi Imperfecti is, of course, obvious. Moreover, the genus Apodachlya in the closely related Leptomitaceae and the genus Blastocladia in the Blastocladiaceae furnish an interesting comparison to the Achlya described above. It is worthy of note that a loss of sexual reproduction is, at least in the forms just mentioned, concomitant with the production of more or less resistant nonsexual spores. This is particularly noticeable in Apodachlya and Blastocladia; since all the known species of these two genera (save perhaps the doubtful *Apodachlya completa* of Humphrey) alike exhibit a consistent production of zoospores and resistant spores with an attendant lack of sexual organs. If the dangerous luxury of theorizing about the evolutionary origin of such structures were permissible, they might be regarded as adaptations evolved to withstand unfavorable conditions in forms that were gradually losing the sexually produced spores that generally serve this purpose. Disregarding the purely theoretical origin of this condition in the Achlya under discussion, however, the actual condition itself remains; and in whatever way this Achlya may be interpreted it at least presents an interesting case of a very distinct, and well defined, but non-sexual representative of a Saprolegniaceous genus usually strongly sexual.

SUMMARY

1. In the method of zoospore production and liberation, and in the character and behavior of the zoospores themselves, the fungus that is the subject of this paper distinctly belongs to the genus Achlya.

2. In contradistinction to most species of the genus, this fungus, as far as observed, entirely lacks sexual reproduction, nor does it produce oogonia and antheridia under the methods of culture usually successful in inducing these organs.

3. The fungus is distinguished by the consistent production, under widely varying conditions, of resistant spores of non-sexual origin and distinct morphological characteristics. These spores differ from the "gemmae" described for other species in their regular occurrence and clearly defined structure.

4. The writer regards this fungus as an Achlya that has lost its sexual reproduction—the resistant function usually assumed by the

spores of sexual origin being in this case taken over by the non-sexual resistant spores.

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U. S. DEP'T OF AGRICULTURE

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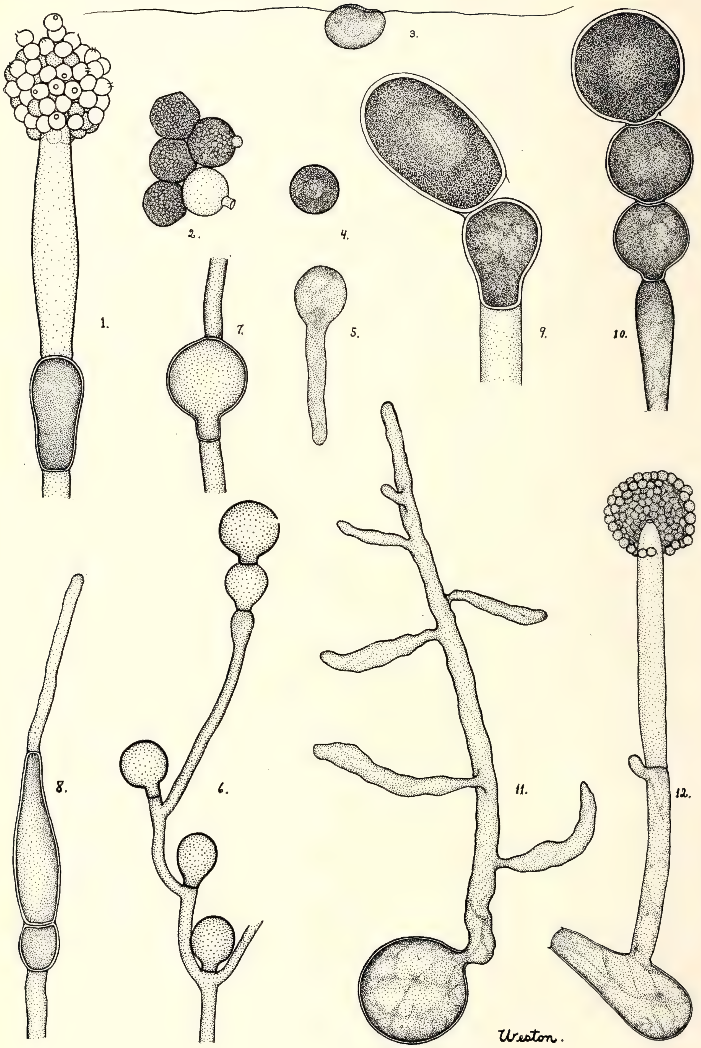
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WESTON: OBSERVATIONS ON ACHYLA.

EXPLANATION OF PLATE XVIII

The figures were drawn from living material at the level of the stage, with the aid of an Abbé camera lucida. The approximate magnification of the combination of lenses used is given in each case, but applies to the original figures, which have been reduced to about ½ their diameter in reproduction.

Fig. 1. Emptied sporangium with hollow sphere of escaped sporangiospores adherent to the tip. Beneath the sporangium a resistant spore has already formed. × 550.

FIG. 2. Group of 5 escaped sporangiospores. From one now empty, a secondary zoospore has already escaped; another shows a papilla of dehiscence forming. × 1,400.

FIG. 3. Laterally biciliate zoospore showing its peculiar shape. The groove from which the cilia arise is above. × 1,400.

FIG. 4. Encysted zoospore after coming to rest. × 1,400.

FIG. 5. Germination of such an encysted zoospore. × 1,400.

FIG. 6. Portion of mature mycelium bearing several resistant spores. × 150.

FIG. 7. Intercalary resistant spore. × 220.

FIG. 8. Two intercalary resistant spores of somewhat irregular shape. × 220.

FIG. 9. Abjunction of a terminal resistant spore by the rupture of its delicate outer wall. × 350.

FIG. 10. Formation of three resistant spores in basipetal series. × 350.

FIG. 11. Germination in nutrient agar of a resistant spore by means of a vigorous germ tube. × 350.

FIG. 12. Germination in pure water of a resistant spore forming a typical Achlya sporangium. Some of the adherent spores have been washed away showing their arrangement in a hollow sphere. × 350.

THE RUSTS OCCURRING ON THE GENUS FRITILLARIA

Charles C. Rees

The Fritillaria rusts of Europe and America furnish an interesting problem for taxonomic study. In the absence of inflorescence the resemblance of the genera Fritillaria and Lilium, upon which occurs *Uromyces Lilii* (Link) Fckl., as interpreted by European authors, is striking. This host resemblance, coupled with the fact that the rusts infecting them present a somewhat similar gross appearance, has resulted in considerable confusion in the naming of many collections.

Evidence derived from a study of types and other material of the Opsis-Uromyces species on these hosts in the Arthur herbarium has led to the conclusion that the species on Fritillaria in Europe is morphologically quite separate and distinct from the rust with the same life-cycle on Lilium in that region and from the American form on the same host as well. The writer is therefore inclined to recognize as a valid species, *Uromyces Fritillariae* (Schlechtd.) Thüm., to which all of the European specimens on Fritillaria, seen by the writer, should be referred. All of the Uromyces collections on Lilium in Europe belong, according to the writer's interpretation, to the species formerly known as *Uromyces Lilii* (Link) Fckl. This species was first described by Strauss[1] under the name *Uredo aecidiiformis* on *Lilium candidum*. Although he used the generic term, Uredo, in designating the form, it is evident from the text that telia are described. For these reasons the species should take the name *Uromyces aecidiiformis* (Strauss). Regardless of the fact that the type of *Uromyces Miurae*[2] Sydow has not been examined, it is the writer's opinion that the American rust on Fritillaria is identical with it. Such disposition of a rust new to this country is not unusual when it is remembered that certain species of rust and other fungi have for a long time been known to be indigenous to Japan and eastern Asia as well as to the western and particularly to the northwestern coast of North America.

[1] Ann. Wett. Ges. **2**: 94. 1811.

[2] Ann. Myc. **11**: 94. 1913.

The morphological differences of these three species are very clearly defined, and the species are easily separated, as shown in the analytical key which follows. Because of its close host relationship, *Uromyces Holwayi* Lagerh.[3] is included in the key. A description of this species, however, is omitted because of the fact that its life-cycle, which includes uredinia, precludes any possibility of its being confused with the species under discussion. No species of Uromyces, other than this one, has yet been reported on Lilium in North America.

Key to Species on Lilium and Fritillaria

Telia exclusively considered

- Teliospore-wall moderately thick (2–3 μ), chestnut-brown.
  - Teliospores globoid (exclusive of apiculus), 23–27 by 31–35 μ. . . . . 1. *Uromyces aecidiiformis.*
  - Teliospores ellipsoid, 18–25 by 29–39 μ. . . . . 2. *Uromyces Holwayi.*
- Teliospore-wall moderately thin (1.5–2 μ), cinnamon-brown.
  - Teliospores narrowly ellipsoid, 14–23 by 24–35 μ, inconspicuously verrucose in lines. . . . . 3. *Uromyces Miurae.*
  - Teliospores ellipsoid to broadly so, 23–31 by 31–42 μ, delicately rugose. . . . . 4. *Uromyces Fritillariae.*

1. **Uromyces aecidiiformis** (Strauss) comb. nov.

Uredo aecidiiformis Strauss, Ann. Wett. Ges. **2**: 94. 1811.
Caeoma Lilii Link in Willd. Sp. Pl. **6**^{2}: 8. 1825.
Caeoma aecidiiformis Schlecht. Linnaea **1**: 239. 1826.
Aecidium Meleagris Duby, Bot. Gall. **2**: 904. 1830.
Erysibe variolosa Wallr. Fl. Crypt. Germ. 195. 1833.
Erysibe rostellata var. *Lilii* Wallr. Fl. Crypt. Germ. 210. 1833.
Uromyces Liliacearum Unger, Einfl. Bodens 216. 1836.
Aecidium Liliacearum Unger, Einfl. Bodens 220. 1836.
Uredo Lilii Rab. Deutschl. Krypt. Fl. **1**: 12. 1844.
Uromyces Rabenhorstii Kunze, Rab. Fungi Eur. *1693a*. 1873.
Uromyces Lilii Kunze, Rab. Fungi Eur. *1693b*. 1873.

O. Pycnia amphigenous, rather numerous, among the aecia, small, 0.1–0.2 mm. across, punctate, conspicuous, subepidermal, honey-yellow, becoming darker later, chestnut-brown-colored ring encircling base, globoid in cross section, 160–190 μ high by 160–190 μ in diameter.

I. Aecia amphigenous, for the most part hypophyllous, also petiolicolous and caulicolous, gregarious in round or elongated groups of various sizes, up to 10 mm. in length, cupulate, low, 0.3–1 mm. in diameter; peridium white, turning to yellowish-brown, opening by a central pore, enlarging later, the margin erect or incurved slightly, erose; peridial cells oblong in cross section, 26–29 by 32–39 μ, abutted, the outer wall 7–13 μ thick, striate, the inner wall 9–12 μ in thickness, very finely and almost inconspicuously verrucose; aeciospores angularly globoid, 19–23 by 19–27 μ; wall pale yellow, 1.5–2.5 μ in thickness, finely and closely verrucose.

---

[3] N. Amer. Fl. **7**: 242. 1907. (As *Nigredo Lilii* (G. W. Clinton) Arth.)

III. Telia amphigenous, numerous, scattered, elongated, 0.2–1.3 mm. in length, tardily naked, finally dehiscent by longitudinal slits in the epidermis, pulverulent, chestnut-brown, ruptured epidermis conspicuous; teliospores broadly ellipsoid to globoid (exclusive of apiculus), 23–27 by 31–35 μ (including apiculus); wall chestnut-brown, about 3 μ thick, a low hyaline apiculus at the apex, moderately rugose with longitudinal parallel ridges, sometimes appearing almost smooth when wet; pedicel very fragile, short, hyaline.

ON LILIACEAE: *Lilium bulbiferum* L., *L. candidum* L., *L. carniolicum* Bernh. and *L. croceum* Chaix. Throughout central Europe.

TYPE LOCALITY: Europe, on *Lilium candidum.*

EXSICCATI: Thüm. Myc. Univ. *1041;* Thüm. Fungi Austr. *848;* Rab. Fungi Eur. *1693;* Sydow, Ured. *1504;* Sydow, Myc. March. *3010;* Kunze, Fungi Sel. *35*.

Even in those collections showing teliospores only, *Uromyces aecidiiformis* is readily distinguished from *Uromyces Holwayi*, since the teliospores of the former are more nearly globoid and have slightly thicker walls than the latter (Fig. 1). However, the presence of aeciospores, which are quite different in the two species, enables one to separate them readily.

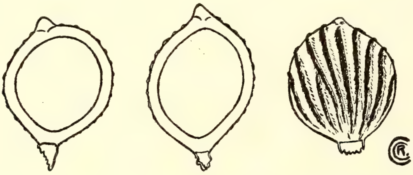

FIG. 1. Teliospores of *Uromyces aecidiiformis* showing optical sections and surface view. × 625.

3. **Uromyces Miurae** Sydow, Ann. Myc. **11**: 94. 1913.

O and I. Pycnia and aecia, unknown.

III. Telia amphigenous, petiolicolous, numerous, occasionally crowded in groups of two or three sori, round or broadly ellipsoid, 0.2–0.7 mm. across, tardily naked, finally dehiscent by longitudinal rents in the epidermis, pulvinate, becoming pulverulent, cinnamon-brown, ruptured epidermis conspicuous; teliospores rather narrowly and irregularly ellipsoid to terete, 14–23 by 24–35 μ, rounded or tapering at apex, usually tapering at base; wall golden to cinnamon-brown, of uniform thickness (1.5–2 μ), a low (1.5–3 μ), hyaline apiculus at apex, moderately and very inconspicuously verrucose, markings arranged to form longitudinal striations, appearing almost smooth when wet; pedicel very short, fragile, a sac-like swelling at point of attachment with spore.

On Liliaceae: *Fritillaria Kamtschatcensis* Ker. Alaska, British Columbia, Japan. *Fritillaria lanceolata* Pursh. Washington.

Type locality: Mt. Shirouma, prov. Shinano, Japan on *Fritillaria Kamtschatcensis.*

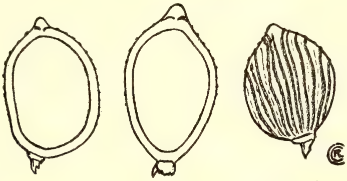

Fig. 2. Teliospores of *Uromyces infrequens* showing optical sections and surface view. × 625.

Distribution: South-central and east-central Washington northwestward through Vancouver and Queen Charlotte islands to southeastern Alaska; also in central Japan.

Exsiccati: Ellis & Ev. N. Am. Fungi *1863.*

It is impossible of course with only the telial stage present to assign this species with certainty to any genus in the classification based on the length of life-cycle, proposed by Arthur.[4] However, in spite of the lack of positive evidence which would indicate its proper taxonomic location, the writer is inclined to consider it an Opsis-form similar in life history to *Uromyces aecidiiformis* and *Uromyces Fritillariae.* Successful cultures or additional material bearing other spore stages will be necessary before the full life history is understood.

---

[4] Eine auf die Struktur und Entwicklungsgeschichte begründete Klassifikation. Result. Sci. Congr. Bot. Vienne 331–348. 1906.

Morphologically this form is notably different from any species yet reported on any Liliaceous host either in North America or Europe and the addition of the North American material extends considerably the distribution of this distinctive species. The teliospores are more narrowly ellipsoid, have considerably thinner walls and are verrucose in longitudinal striations (Fig. 2). The teliospores of the other species discussed in this paper are distinctly rugose.

4. **Uromyces Fritillariae** (Schlechtd.) Thüm.; Voss, Oesterr. Bot. Zeits. **26**: 297. 1876

Caeoma Fritillariae Schlecht. Linnaea **1**: 240. 1826.

O. Pycnia amphigenous, rather numerous, scattered among the aecia, small, punctiform, conspicuous, subepidermal, honey-yellow becoming dark chestnut-brown, flattened globoid in cross section, 80–95 μ in width by 60–80 μ high; ostiolar filaments free.

I. Aecia amphigenous, caulicolous, petiolicolous, crowded in linear groups, cupulate, low, 0.3–1 mm. in diameter; peridium at first white, becoming yellowish-brown later, opening by a central pore after a longitudinal splitting of the epidermis has taken place, the margin incurved, erose; peridial cells oblong in cross section, 19–26 by 29–32 μ, abutted, the outer wall 9–13 μ thick, striate, the inner wall 9–12 μ thick, very finely verrucose; aeciospores angularly globoid to ellipsoid, 16–22 by 21–28 μ; wall yellow, 2–3 μ in thickness, very finely, closely and almost inconspicuously verrucose.

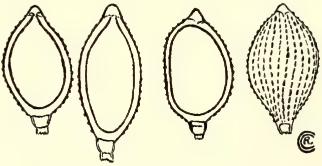

FIG. 3. Teliospores of *Uromyces Fritillariae* showing optical sections and surface view. × 625.

III. Telia amphigenous, caulicolous, petiolicolous, numerous, scattered, elliptical, rather small, 0.1–0.8 mm. in length, tardily naked, dehiscent by longitudinal slits in the epidermis, somewhat pulverulent, chestnut-brown, ruptured epidermis conspicuous; teliospores broadly ellipsoid to obovoid, 23–31 by 31–42 μ; wall golden- to cinnamon-brown, 1.5–2 μ thick, low (1.5–3 μ) hyaline apiculus at apex, rather delicately rugose in longitudinal striations; pedicel hyaline, short, fragile.

ON LILIACEAE: *Fritillaria Meleagris* L. in Europe.

TYPE LOCALITY: Southern Europe, on *Fritillaria Meleagris.*

EXSICCATI: Sydow Ured. *107;* Thüm. Myc. Univ. *553*, *728;* Roum. Fungi Gall. *2922.*

This species differs in many respects from *Uromyces aecidiiformis* with which it has been included by European authors. The teliospores (Fig. 3) are larger, thinner walled and less prominently rugose than those on Lilium.

Grateful acknowledgment is due Dr. J. C. Arthur for the unrestricted use of his herbarium material upon which this study is based; to Professor H. S. Jackson, for his many helpful suggestions, the writer is also deeply indebted.

PURDUE UNIVERSITY,
AGRICULTURAL EXPERIMENT STATION,
WEST LAFAYETTE, INDIANA

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CONTENTS

Fertility in Cichorium intybus: the sporadic occurrence of self-fertile plants among the progeny of self-sterile plants . . . . . A. B. STOUT 375
Inheritance of endosperm color in maize . . . . . ORLAND E. WHITE 396
The influence of light and chlorophyll formation on the minimum toxic concentration of magnesium nitrate for the squash. R. B. HARVEY and R. H. TRUE 407
The use of the vibration galvanometer with a 60-cycle alternating current in the measurement of the conductivity of electrolytes. NEWTON B. GREEN 411
Immunochemical studies of the plant proteins: proteins of the wheat seed and other cereals. Study IX. R. P. WODEHOUSE 417
The toxicity of galactose and mannose for green plants and the antagonistic action of other sugars toward these . . . . . LEWIS KNUDSON 430

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FERTILITY IN CICHORIUM INTYBUS: THE SPORADIC OCCURRENCE OF SELF-FERTILE PLANTS AMONG THE PROGENY OF SELF-STERILE PLANTS

A. B. STOUT

The writer (1916) has already presented the evidence that the very prevalent self-sterility (and cross-sterility as well) in chicory can be ascribed to a *physiological incompatibility* operating between sex organs or sex cells that are fully formed, anatomically perfect, potentially functional and of simultaneous development. It was noted that this type of sexual sterility is sharply to be distinguished from sterility due to *anatomical incompatibility* (more or less purely structural differences and adaptations such as hercogamy), *impotence* (failure to produce gametes) or *embryo abortion* (death of egg after fertilization or death of young embryo). I also at that time discussed and summarized the literature bearing on such phenomena.

From my studies made in 1912 and 1913, it appears (Stout 1916, p. 365–366) that self-sterility is the rule in chicory. Three plants (designated *A*, *B*, and *C*) of wild stock were found to be self-sterile, as were 52 plants grown from the open fertilized seed of these plants, and all plants tested of ten cultivated varieties were self-sterile. In one variety (Barbe de Capucin), 29 plants of one planting and 5 of another were tested, and of other varieties about five plants of each were tested. The total of about 135 plants from these various sources were self-sterile.

However, in the pedigreed cultures grown in 1913, a few plants exhibiting varying degrees of self-fertility appeared quite sporadically among the F_1 progeny of various crosses between self-sterile plants. Of the 75 plants derived by crossing plant *A* with plant *E22* (of the variety Barbe de Capucin), only eight were self-fertile. Of 21 plants,

[The **Journal** for June (**4**: 315–374) was issued June 29, 1917]

the offspring of *A* and *E3*, four were self-fertile. Seventeen plants, the offspring of *C* and *E3*, were self-sterile, as were 30 plants from seed of a cross between a white-flowered plant, $(A \times C)$ *no. 1*, of wild stock and a plant of the variety "improved striped-leaf." The number of self-fertile plants, therefore, varied greatly in the different series, but in no series was the proportion very large.

The self-fertile plants mentioned above appeared after only one generation of ancestry known to be self-sterile. Furthermore, the parents in each cross were not closely related and were somewhat different in vegetative habit and flower color. As previously recognized (1916, p. 415), these results raised some question regarding the influence of wide-crossing as compared with that of inbreeding on the development of self-compatibilities, especially as continued inbreeding in the variety "red-leaved Treviso" had in two generations given only one feebly self-fertile plant out of a total of 49 plants (complete data given in 1916, Table 7).

In order to obtain further data on this question, it was planned to continue inbreeding within this variety, increasing the number of plants grown in 1916, and at the same time to grow for comparison an F_1 generation from crosses between plants of this variety and a self-sterile plant of a wild stock. The present paper will deal especially with the data obtained from these cultures.

Description of the Cultures

The variety "red-leaved Treviso" is a cultivated salad chicory that has been developed in continental Europe. As grown for commercial seed production the variety is biennial, seed being sown one summer for a crop that matures in the following summer. As grown in my culture the plants are more nearly annual. Seed is sown in January in flats, and the seedlings are potted and kept in continuous growth in the greenhouse until spring, when they are planted in the field. Under such treatment the plants, as a rule, reach full development in the following August. The general habit of growth of the mature plants is well shown in text-figure 1. The height has ranged from 4½ to 6½ feet with the greater number of plants about 5 feet tall. The plants are rather sparsely branched near the base but rather abundantly branched above. In the early stages of growth the rosette leaves are numerous, of large size, and erect. One of the marked characteristics of the family I have grown is the development of a type of fasciation

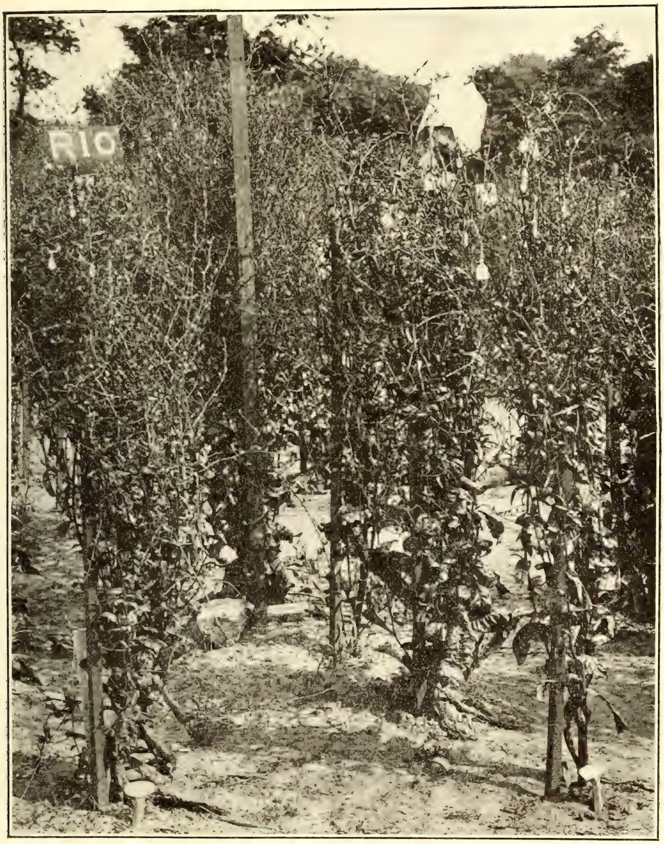

FIG. 1. Typical plants of the variety red-leaved Treviso. The marker stands by self-fertile plant (R. Ser. 10, No. 8); all other plants in the view were self-sterile. From photograph taken in the afternoon when all flower heads are closed.

involving duplication and cohesion of the main axis. Two main stems develop with a single root system. Occasionally these are separated from the root upward, but most usually the two are more or less fused for a distance, the fusion finally becoming complete near the top of the plant. None of the plants of this variety have shown any tendency to live over winter. The maturity and death of the stems and branches is accompanied by death of the roots. Several attempts to obtain new plants from root cuttings taken at the time of the maturity of plants have failed.

The wild white-flowered plant used in crosses with plants of the red-leaved Treviso is perennial as are wild plants of chicory in general. In the five years it has been under observation its mature height has ranged from 2¼ to 2½ feet. Its rosette leaves are few, much smaller in size than those of the red-leaved Treviso, and are flat in habit of growth. The branches are few and strongly horizontal, giving the plant a sparsely branched and scraggly appearance.

The F_1 generation plants of the crosses between plants of the red-leaved Treviso variety and the wild plant just mentioned were more like the red-leaved Treviso in habit of growth. They were all blue-flowered. Their height ranged from 4 to 6 feet, and they were abundantly and profusely branched from the base. The degree of the duplication of the main stem was much less than in the family of the red-leaved Treviso. As shown in text figure 2, the plants of this hybrid generation were large and well developed and of marked vegetative vigor. They were far more robust and vigorous in growth than the wild parent, and in respect to the degree of branching they were more developed than plants of the red-leaved Treviso strain.

The sex vigor of these plants and of plants of the Treviso variety in respect to *production* of flowers was commensurate with the vegetative vigor. From statistical data obtained in studies of flower number, it was found that the total number of flower heads produced by individual plants ranged roughly from 2,000 to 3,500 with the average number of flowers per head at about 17. At the climax of development as many as 100 to 150 flower heads opened in a single day. These statements together with the descriptions given and the illustrations in the accompanying text-figures give some conception of the full and complete sex vigor seen in the profuse production of flowers that set seed when pollinated with pollen that was compatible.

It will also readily be observed that the inbreeding within the family of red-leaved Treviso involved crosses between plants of close blood relationship and of decided similarity, and that the fertilization in

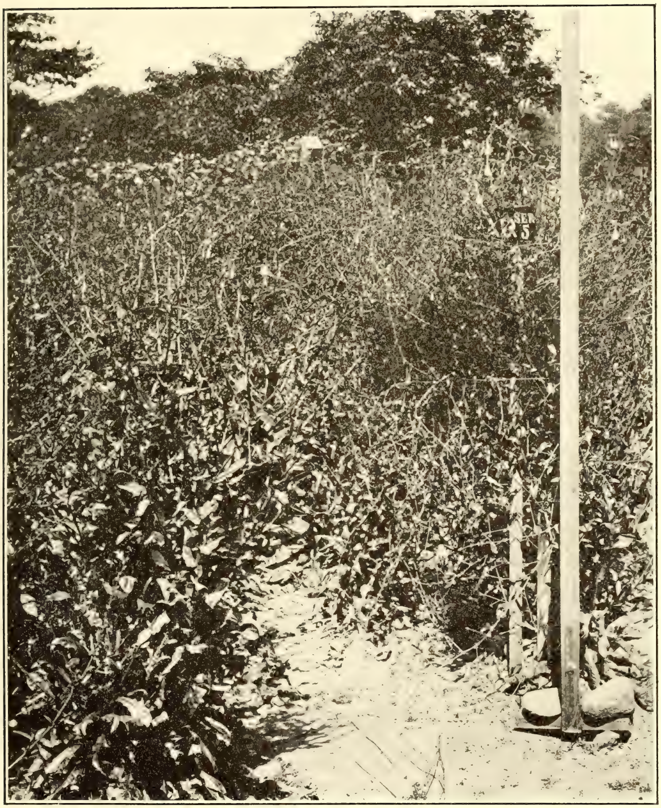

FIG. 2. Typical plants of the F_1 generation of the crosses between plants of the Treviso variety and the wild plant A.

these plants with pollen from the wild plant *A* constitutes a comparatively wide cross both in respect to blood relationship and to the vegetative characteristics of the respective parents.

Results of the Self-Pollinations of the 1916 Crop of "Improved Red-Leaved Treviso"

A total of 103 plants were grown in this crop. All were descended from two plants of the 1913 crop, and all but two had three generations of parentage known to be self-sterile. The data for the self-pollinations made on these plants are given in Table 1. Here the plants are grouped, as they were grown, in series according to the immediate parentage. The table gives the total number of heads upon which controlled self-pollinations were made, the number of heads producing no seed, the number of heads with seed, the number of seeds per head, and the percentage of fertility. Frequently birds ate seeds, indicated in the tables by " B," and thus interferred somewhat with the determination of the percentage of fertility.

The 10 sister plants of Ser. 7 were all self-sterile; of the 19 sister plants of Ser. 8, one was feebly self-fertile; of the 25 plants of Ser. 9, three were self-fertile; of Ser. 10, one of 10 plants was self-fertile; of Ser. 11, five out of 19 were self-fertile; and one of the 18 plants of Ser. 12 was self-fertile. Of the total 101 plants descended from self-sterile parentage, 11 were self-fertile in some degree. With the exception of Ser. 7, one or more self-fertile plants appeared in each series. It may be noted that three self-fertile plants, two of which were rather highly self-fertile, appeared in Ser. 9, which was derived by crossing two sister plants of the previous generation. This series was from a more closely inbred parentage than were the other series.

The total number of flower heads pollinated in these series is 1205. As a rule, not less than 10 heads were pollinated on a plant, and in nearly all cases this total includes pollinations made on several different days. When it became evident that some plants were self-fertile, special efforts were made to continue self-pollination on them in order to secure an abundance of seed for future progeny. However, as shown in the table, the number of heads pollinated on plants completely self-sterile is also often high.

The degree of self-fertility, judged by the percentage of flowers setting seed, varied considerably. Most of the self-fertile plants were feebly self-fertile, producing as a rule only a few seed per head in only a few of the heads manipulated. Others, as no. 34 of Ser. 9 and no. 8 of Ser. 10, set seed in every head pollinated, and in numerous heads the numbers were nearly equal or even equal to all that were

TABLE 1

Record in 1916 for Self-pollinations of the Cultures of "Improved Red-leaved Treviso"

	Record for Heads Pollinated				Fertility (%)
	Total No. Heads.	With No Seed	With Seed	Seeds per Head. Remarks	
Series 6 ...				Parentage, Series 4, no. 21. A self-fertile plant	
No. 3 ...	10	10	0		
" 4 ...	15	15	0		
Series 7....				Parentage, Ser. 1, no. 8×Ser. 5, no. 5.	
No. 1....	14	14	0		
" 2....	15	15	0		
" 3....	20	20	0		
" 8....	10	10	0		
" 10....	13	13	0		
" 12....	11	11	0		
" 17....	7	7	0		
" 19....	10	10	0		
" 20....	10	10	0		
" 21....	10	10	0		
Series 8....				Parentage, Ser. 4, no. 12×Ser. 5, no. 8.	
No. 1....	15	15	0		
" 2....	10	10	0		
" 3....	13	13	0		
" 4....	14	14	0		
" 5....	14	14	0		
" 7....	13	10	3	1, 2, 10.	0.06
" 8....	12	12	0		
" 10....	12	12	0		
" 11....	11	11	0		
" 12....	14	14	0		
" 13....	10	10	0		
" 14....	10	10	0		
" 15....	11	11	0		
" 16....	12	12	0		
" 17....	12	12	0		
" 18....	12	12	0		
" 19....	21	21	0		
" 20....	10	10	0		
" 22....	10	10	0		
Series 9....				Parentage, Ser. 5, no. 1×Ser. 5, no. 6.	
No. 1....	10	10	0		
" 2....	12	12	0		
" 3....	15	15	0		
" 6....	27	1	26	1, 4, 6, 6, 6, 7, 7, 7, 8, 8, 9, 9, 9, 9, 10, 10, 10, 11, 11, 11, 11, 12, 13, 13, 13, B	0.50
" 7....	10	10	0		
" 10....	14	14	0		
" 12....	14	14	0		
" 13....	10	10	0		
" 19....	12	12	0		
" 20....	10	10	0		
" 21....	5	5	0		
" 25....	24	24	0		
" 26....	12	12	0		

Table I—*Continued*

	Record for Heads Pollinated				Fertility (%)
	Total No. Heads	With No Seed	With Seed	Seeds per Head. Remarks	
No. 28....	9	9	0		
" 29....	10	10	0		
" 30....	15	15	0		
" 31....	10	1	9	B, B, B, 1, 2, 2, 2, 3, 4	0.13
" 32....	12	12	0		
" 34....	23	0	23	3, 6, 7, 9, 9, 10, 10, 10, 10, 10, 11, 12, 12, 12, 13, 13, 14, 14, 14, 14, 14, 15, 18	0.71
" 37....	9	9	0		
" 38....	5	5	0		
" 40....	10	10	0		
" 42....	15	15	0		
" 43....	10	10	0		
" 47....	13	13	0		
Series 10....				Parentage, Ser. 4, no. 12×Ser. 5, no. 9	
No. 1....	12	12	0		
" 2....	17	17	0		
" 3....	10	10	0		
" 4....	10	10	0		
" 5....	9	9	0		
" 6....	9	9	0		
" 7....	20	20	0		
" 8....	24	0	24	4, 6, 8, 8, 8, 9, 9, 9, 9, 10, 10, 11, 11, 11, 12, 12, 12, 12, 13, 13, 14, 14, 15, 16, 17	0.63
" 10....	11	11	0		
" 11....	8	8	0		
Series 11....				Parentage, Ser. 5, no. 1×Ser. 1, no. 8	
No. 1....	13	13	0		
" 4....	14	14	0		
" 8....	11	11	0		
" 11....	10	10	0		
" 12....	12	10	2	1, 5	0.03
" 16....	9	9	0		
" 24....	8	3	5	1, 1, 3, 4, 5	0.11
" 25....	14	14	0		
" 27....	11	11	0		
" 28....	10	10	0		
" 29....	10	8	2	2, 4	0.04
" 31....	10	10	0		
" 32....	12	9	3	1, 1, 3	0.03
" 35....	8	8	0		
" 37....	18	17	1	12	0.04
" 40....	10	10	0		
" 43....	11	11	0		
" 44....	11	11	0		
" 49....	8	8	0		
Series 12....				Parentage, Ser. 5, no. 9×Ser. 4, no. 18	
No. 1....	10	10	0		
" 2....	10	10	0		
" 3....	12	12	0		
" 4....	12	12	0		
" 5....	11	11	0		

Table I—*Continued*

	Record for Heads Pollinated				Fertility (%)
	Total No. Heads	With No Seed	With Seed	Seeds per Head. Remarks	
No 6....	9	9	0		
" 7....	10	10	0		
" 8....	13	13	0		
" 9....	12	12	0		
" 10....	10	10	0		
" 11....	13	0	13	B, B, 3+B, 3+B, 3+B, 5+B, 7, 8, 10, 10, 11, 18, 22	0.73?
" 12....	10	10	0		
" 13....	12	12	0		
" 16....	10	10	0		
" 17....	11	11	0		
" 18....	10	10	0		
" 19....	13	13	0		
" 20....	5	5	0		

possible. It is not to be considered that the degree of fertility is absolutely determined, and especially in those cases when birds (Ser. 12, no. 11) ate all or a part of the seed produced in certain heads. The detailed data, however, make it quite clear that various degrees of self-compatibility may exist. The evidence in this particular is quite identical with that already reported in 1916. In Ser. 11 a comparatively large proportion of plants, 5 out of 19, were self-sterile, but the fertility was low in each case.

The two plants of Ser. 6 were derived from self-fertilized seed of a plant that was feebly self-fertile and which was the only self-fertile plant that appeared in my crops of this variety previous to 1916. The two plants were self-sterile.

Self-Compatibilities and Incompatibilities among Plants of the 1916 Crop of F_1 Generation Obtained by Crossing a Plant (*A*) of Wild Stock with Plants of the Variety "Red-Leaved Treviso"

The data for the self-pollinations of this generation are presented in Table 2. The wild white-flowered plant *A* was the pollen parent for Series 1–4 and the seed parent of Series 5. Five different plants of the 1915 generation of "red-leaved Treviso" were concerned in the parentage, as indicated in the table. The uncertainties of securing compatible cross-pollinations among self-sterile plants (Stout, 1916,

Table 2

Data for Self-pollinations of F_1 generation Derived by Crossing a Wild White-flowered Self-sterile Plant (A) With Self-sterile plants of the Variety "Red-leaved Treviso"

Plant.	Data for the Heads Pollinated				Fertility (%)
	Total No. Heads	Heads With No Seed	Heads With Seed	Seeds per Head. Remarks	
RA. Ser. 1.				Parentage, R. Ser. 1, no. 7×A	
No. 1.	10	10	0		
" 2.	10	6	4	1, 1, 1, 5	0.05
" 3.	11	11	0		
" 4.	10	10	0		
" 5.	11	11	0		
" 6.	12	12	0		
" 7.	14	14	0		
" 8.	10	10	0		
" 9.	10	10	0		
" 10.	12	12	0		
" 11.	10	10	0		
" 12.	11	11	0		
" 13.	12	12	0		
RA. Ser. 2.				Parentage, R. Ser. 1, no. 2×A	
No. 1.	12	12	0		
" 2.	15	15	0		
" 3.	15	15	0		
" 6.	13	13	0		
" 7.	14	14	0		
RA. Ser. 3.				Parentage, R. Ser. 1, no. 6×A	
No. 1.	10	10	0		
" 2.	11	11	0		
" 3.	11	11	0		
" 4.	11	11	0		
" 5.	12	12	0		
" 7.	12	12	0		
" 8.	15	15	0		
" 9.	11	11	0		
" 10.	17	17	0		
" 13.	10	10	0		
" 14.	10	10	0		
" 15.	12	12	0		
" 16.	12	12	0		
" 17.	11	11	0		
" 18.	16	16	0		
" 19.	11	11	0		
" 20.	11	11	0		
" 21.	16	16	0		
" 22.	10	10	0		
" 23.	11	4	7	1, 3, 4, 5, 6, 7, 11	0.19
" 24.	12	12	0		
RA. Ser. 4.				Parentage, R. Ser. 5, no. 16×A	
No. 1.	10	10	0		
" 2.	10	10	0		
" 3.	10	10	0		
" 4.	19	19	0		

TABLE 2—*Continued*

Plant	Data for the Heads Pollinated				Fertility (%)
	Total No. Heads	Heads With No Seed	Heads With Seed	Seeds per Head. Remarks	
No. 5.	12	12	0		
" 6.	15	15	0		
" 8.	10	10	0		
" 9.	14	14	0		
" 10.	10	10	0		
" 11.	13	13	0		
" 12.	10	10	0		
" 13.	10	10	0		
" 14.	12	12	0		
" 15.	11	11	0		
" 16.	10	10	0		
AR. Ser. 5.				Parentage, A×R. Ser. 1, no. 1	
No. 1.	10	10	0		
" 2.	11	11	0		
" 3.	10	10	0		
" 4.	10	10	0		
" 5.	10	10	0		
" 6.	10	10	0		
" 7.	10	10	0		
" 8.	12	12	0		
" 9.	12	12	0		
" 10.	10	10	0		
" 11.	10	10	0		
" 12.	13	13	0		
" 13.	12	12	0		
" 14.	12	12	0		
" 15.	13	13	0		
" 16.	9	9	0		
" 17.	12	12	0		
" 18.	8	8	0		
" 19.	12	12	0		

Table 14) made it somewhat difficult to limit the parentage of the various series to the same parents, which would, of course, be highly desirable. Thus it happens that the immediate parents of these series are not the same as those of the series reported in Table 1; the plants involved are, however, closely related sister plants.

In making the cross-pollinations between the self-sterile parents here involved, no attempts were made to emasculate or to depollinate the seed parent. In brushing a flower head of a prospective seed parent with a flower head from a plant selected for a pollen parent, there was necessarily a full and rather thorough mixture of the two lots of pollen with apparently an equal chance that both should be distributed on stigmatic surfaces. A total of 54 plants were derived from four different seed parents of the red-leaved Treviso (Ser. 1–4, Table 2) and 19 were derived from the wild white-flowered plant as a seed parent. All these plants were unmistakably hybrids. In no case did a plant's own pollen function in fertilization.

It may be noted here that East (1915) has made the suggestion that the physiological conditions operating in self-incompatibility involve a failure on the part of the plant's own pollen to stimulate the proper secretions in its pistil necessary for growth of the pollen tubes. If this were the case, it would seem that self-sterility might be removed, in part at least, by mixing pollen as I have done in the crosses referred to above. Such, however, was not the result. It is possible that such results might more readily be obtained in species in which the fertilization processes are much less rapid than in chicory.

Of the 73 plants of this F_1 generation, only two plants were self-fertile with percentages of 5 and 19. In only two cases were the number of heads pollinated less than 10. The results are therefore very decided. All of these plants were blue-flowered and were quite similar in general vegetative habit and appearance. All flowered profusely throughout the season, and, as is the case with plants having only this type of sterility (physiological incompatibility), all set abundant seed in many heads open-pollinated.

Cross-Incompatibilities among the Plants Grown in 1916

A brief summary may here be given regarding the results of cross-pollinations made during 1916. Of the cultures of red-leaved Treviso (R), 37 different plants were tested in a total of 34 different combinations; of these 16 were sterile and 18 fertile in some degree. Among the plants of the F_1 generation (RA), 24 combinations of cross-pollinations were made involving 37 different plants. Of these 9 were sterile and 15 were fertile in some degree. As indicated by the figures, the combinations among the R plants involved fewer plants proportionally and more reciprocals than did those among the RA plants. No particular emphasis can be given to the number here obtained in its bearing on the influence of inbreeding or cross-breeding. The data obtained from these plants selected at random, however, indicate that cross-incompatibilities exist in marked degree. The results in this respect are quite in agreement with those already reported (1916, Tables 9–14), not only for the red-leaved Treviso but for other cultures of chicory.

Discussion and Conclusion

The sporadic development of self-compatibility giving self-fertility among the progeny of self-sterile lines of descent is in decided evidence in the cultures reported above. No doubt if a larger number of the "red-leaved Treviso" variety had been grown and tested, more than one self-fertile plant would have been found previous to the crop of 1916. However, they were not found and the variety was kept in pedigreed cultures by crossing self-sterile plants.

Self-compatibility is therefore a characteristic that was new in expression, at least to the particular and immediate line of descent involved. A total of 101 plants of the 1916 crop had three generations of ancestry known to be self-sterile; of these 11 plants were self-fertile.

There is, therefore, much in the occurrence of these plants that suggests discontinuous variation or mutation. However, the fertilities of these self-fertile plants vary. They grade over to complete self-sterility. The variation in the self-fertility of plants grown from self-fertile parents (Stout, 1916, Table 6) is much more continuous and is indicative that the irregular and somewhat discontinuous variation seen in the intensity of fertilities is only an apparent one due to the few cases observed.

It is to be noted that there have been scarcely any attempts made to study the progeny of self-sterile plants in species and varieties known to be strongly self-sterile by continued inbreeding in pedigreed lines of descent. Compton (1912, 1913) has reported that in *Reseda odorata* "self-sterile plants when bred *inter se* throw self-sterile offspring only," but he has not published data regarding the number of such families, the number of plants, or the number of generations tested. East (1915) has reported that the inter-specific hybrids between *Nicotiana forgetiana* and *N. alata grandiflora* have been completely self-sterile for four generations, and that a total of over 500 plants were tested. Data on the behavior of the parent plants, or even of the two parent species, were evidently not obtained. Correns (1912, 1913) was especially interested in the study of cross-incompatibilities and evidently tested the self-fertility of only 13 of the total of 60 sister plants obtained by crossing two self-sterile plants of *Cardamine pratense*. Of these, however, three plants appear to have been self-fertile.

In view of the prevalence of self-incompatibilities in many plants of economic importance, such as cabbage, rye, apple, plum, prune, cherry, blueberry, etc., it is somewhat surprising that more searching studies have not been made on the sporadic occurrence of self-fertile plants. It is somewhat in doubt, therefore, whether there exists a species, a variety, or even a strain of plants in which self-sterility due to physiological incompatibility is absolute. However, such may exist especially among certain hybrid strains as is suggested by East's data. Many further data are needed to allow of any adequate statement of the various degrees and intensities of such self-sterility in species or in different strains as a whole. The general evidence, however, suggests that in many such cases the sporadic occurrence of self-fertile plants may be quite as it is in chicory.

The almost complete self-incompatibility of the F_1 progeny of the crosses between plants of the "red-leaved Treviso" variety and the wild white-flowered plant *A* is noticeable. The occurrence of only two self-fertile plants of feeble fertility out of 73 such plants emphasizes the sporadic nature of the development of self-compatibility. This may also be considered as evidence that wide crossing inside the species does not especially favor the development of self-compatibility. In fact, a comparison of the behavior of these plants with that of the 1916 crop of the inbred plants of the "red-leaved Treviso" variety leads to the conclusion that inbreeding is more favorable to the development of self-compatibility than is wide crossing. In East's results (1915) all plants tested, some 500 in number, of the F_1, F_2, F_3, and F_4 generations were found to be self-sterile. As these were the offspring of an interspecific cross, it does not seem that wide crossing has here favored the development of self-compatibility. It should be noted that East suggests that there may be some increase in the development of cross-incompatibilities among the later generations, which he considers may be due to an increased homozygosity, but the evidence is not conclusive on this point.

I have not sufficient data to judge adequately of the frequency of cross-incompatibilities among the various series and generations of chicory grown. Cross-incompatibility has occurred in each generation of the red-leaved Treviso variety (for data obtained in 1914 and 1915 see Stout, 1916, Table 14) as it has in all other families thus far tested (Stout, 1916, Tables 9–13). Everywhere that I have tested for cross-incompatibility in chicory it has been found to be very general and to exist in various grades of intensity.

The numbers of self-fertile plants which appeared among the F_1

generation of crosses between the wild plant *A* and plants of the cultivated common chicory (*E* Series) are somewhat higher than those of the F_1 generation (RA plants) derived by crossing this same wild plant with plants of the red-leaved Treviso here reported. The strain (E) has not, however, been inbred in pedigreed cultures as has the red-leaved Treviso strain, so there are less adequate data on the comparative value of inbreeding and crossing with this variety.

The character of physiological self-compatibility giving fertility appears in a very irregular and sporadic manner, and it exists in different degrees of intensity in different plants. It has appeared in chicory in a family of the variety known as red-leaved Treviso after three generations of self-sterile ancestry and no doubt would occur with equal irregularity and intensity after many generations of such ancestry. It seems very conclusive therefore that the causes of self-incompatibilities are not to be ascribed to a similarity of nuclear constitution involving definite hereditary units of germ plasm which either directly determine incompatibilities (especially Correns's view of line-stuffs) or which indirectly determine them (East's view). Furthermore, the variability of the offspring grown from self-fertile plants in chicory shows a very irregular inheritance of the characteristic of self-compatibility and makes it quite clear that the expression of self-compatibility is quite of the nature of a fluctuating variability, and that self-compatibility and self-incompatibility, in chicory at least, are not to be described in terms of dominant and recessive characters which behave in any sort of Mendelian manner.

The evidence seems conclusive that the actual conditions giving the various grades of self-compatibility, and of self-incompatibility (undoubtedly there are various grades of incompatibility giving complete sterility) as well, are decidedly individual. Various aspects of this question in relation to conceptions of fertilization and to the phenomena of serum incompatibilities have already been discussed (Stout, 1916). It must be remembered that a plant whose two sets of sex-organs are completely incompatible is itself derived from the fusion of two cells that were compatible. The interactions between pistil and pollen-tubes were compatible. The germ plasms of the two sex cells were compatible in fusion, in the somatic life of the diploid cell structure of the resulting individual, and in the more intricate interactions involved in sporogenesis occurring in that individual. Yet in cases of complete self-incompatibility none of the pollen grains are functional on the pistil of the plant.

Such conditions emphasize the marked individuality of the development of conditions giving incompatibility. The conditions are fundamentally physiological and arise apparently in connection with the differentiation of the two sets of so-called sex organs. Important to an understanding of the facts of differentiation here involved are the phenomena of cross-incompatibilities. Three sister sporophytes which are quite identical in all vegetative characters may possess sex organs that are incompatible to the extent that complete self-sterility is in evidence; no. 1 may be incompatible with the male sex organs (microgametophytes and gametes) of no. 2, but compatible with those of no. 3. This difference in relation is certainly indicative of differences in the physiological qualities of the two lots of male gametophytes. Conversely the microgametophytes and gametes produced by a single sporophyte may act quite differently on the female sex organs borne on two other sporophytes, being compatible in one case and incompatible in the other. This indicates, likewise, a difference in the condition of the two sets of female organs (including pistils). Furthermore, the data as to the occurrence of cross-incompatibilities in chicory even indicate that reciprocal crosses between two plants may give quite the opposite results, showing that the relations of the two sets of sex organs may not be interchangeable.

In such phenomena we may recognize a loss of sex-vigor which is concerned with the function of gametophytes and gametes. The decrease in fertility is entirely independent of a decrease in the production of spores. Furthermore, there appears to be full and complete development of the macrogametophyte and its egg; its development is certainly not inhibited by the condition of the pistil in which it develops. There is no evidence that the microgametophyte is not fully developed with reference to its differentiation. Although often involving a decreased vegetative growth of the pollen-tube, the inhibition appears fundamentally to involve function.

The reactions involved in self- and cross-incompatibilities do seem to involve, to some extent at least, as Jost (1907) and East (1915) have especially emphasized, an interaction between the haploid pollen-tube and the diploid tissues of the pistil. There may be some question as to what extent these relations are involved.

Incompatibilities are evidently indicated not only by an inability to produce embryos, but also sometimes by a feeble viability of those that are produced. This death of embryos among seed produced by the self-pollination of different sister plants is quite as fluctuating in degree as is the production of seed itself. In its effect it is often quite like the conditions observed in the "zygotic sterility" which Davis (1915*a*, 1915*b*, 1916) has observed in the Oenotheras, especially those of hybrid origin. In chicory, however, the noticeable failure in seed production suggests that much of the embryo abortion observed may also involve a sort of sexual incompatibility. Embryo abortion, however, may be due purely to conditions of nutrition, especially in those species which exhibit no physiological incompatibility.

The incompatibilities in chicory are obviously not purely a question of haploid against diploid, but of a particular kind of haploid and diploid relationship. In discussing these various points, the writer (1916, p. 436–440) has pointed out that our knowledge of the physiology of pollen-tubes is scarcely sufficient to decide whether the critical point in the growth of the pollen-tube is determined by purely nutritive reactions with the pistil as such or whether it is really determined by the diffusion of secretions (hormones) from the macrogametophyte. The writer hopes to be able to state later somewhat definitely from cytological investigation what the relative developments and nuclear phenomena in chicory are.

In discussing the various aspects of the relation of cell organization to the development of compatibilities and incompatibilities, the writer (1916, p. 416) has pointed out that the role of any particular combination of germ plasm elements, as far as can be judged by their expression as characters in parents, in sister plants and in offspring, must be quite secondary as far as incompatibilities are concerned to a more general quality of the tissue and cell organization that develops in connection with ontogenetic growth and development. The conceptions of Jost (1907), Morgan (1904, 1910), and East (1915) are fundamentally based on this same generalization as I there pointed out.

Much the same idea, if I understand their position aright, has since been expressed by Goodspeed and Clausen in stating that such cases of physiological incompatibility seem to involve "non-specific" disturbances in the "reaction systems" (germ plasm) (1917*a*, p. 46). These authors have embodied in the conception of "reaction systems" (1916, 1917*a*) a view which in some measure is a revolt against the extreme formalism of the Mendelian factorial hypothesis, and in this sense the conception is useful in the interpretation of the phenomena of sterility especially of the type I have called impotence. In their application of this conception to the almost complete impotence of the F_1 hybrids of *Nicotiana Tabacum*×*N. sylvestris*, they are dealing with the well-known cases of degeneration so often observed during sporogenesis in interspecific hybrids. They believe that the very few perfect spores formed represent the *Tabacum* and *sylvestris* extremes of a combination series. In other words, these few spores represent the cases where the parental germ plasms segregated without mutual influence. The greater number of recombinations, however, were incompatible combinations of various elements derived from the two germ plasms. There are very few of the two original combinations that survive reduction and sporogenesis. In somatogeneses the incompatibility is seen, they believe, in a complete dominance of the *Tabacum* characters (1717*a*, 1917*b*). Whether involving chemical or mechanical reactions or involving differences in developmental tendencies in the sense used by Tischler (1907), (Stout, 1916, p. 423–427) such intra-cellular incompatibilities arise especially in the reorganization of cells during or immediately following reduction as has long been known.

In the case of physiological incompatibility, as in chicory, there appears to be no impotence except of a purely accidental sort. Any recombination system may survive, and in chicory sporogenesis in the offspring of crosses between the red-leaved Treviso variety and a wild white-flowered plant must, it would seem, give many new recombinations. The range of these recombinations must be quite the same in the various sister plants both of the F_1 generation hybrids and of the various series of red-leaved Treviso. Yet for the self-sterile plants, and these are here in greater number, all the pollen grains fail to function irrespective of the character of the particular germ plasm organization from which they came and of which they may be variously composed. On the other hand in the self-fertile plants that are sister plants of such self-sterile plants, germ cells of much the same hereditary constitutions (as judged by the characters of the plants that bear them) are compatible.

Furthermore, in the cases of self-fertility of any degree (or cross-fertility as well), the evidence thus far obtained from hybrid generations does not indicate that the fertilizations involved selective or preferential mating which favored fusion between particular recombinations of germ plasm with respect to hereditary characters.

The determination of whether physiological self- and cross-incompatibilities giving sterility involve similarity or dissimilarity of constitutional organization is, of course, very fundamental to the understanding of the nature of fertilization. Although rather widely differing in particular applications, the conceptions advanced as to the causes of physiological sexual self-incompatibility in such hermaphrodite plants as *Eschscholtzia* (Darwin, 1877), *Cardamine* (Correns, 1912, 1913), *Reseda* (Compton, 1912, 1913), *Nicotiana* (East, 1915), and in such hermaphrodite animals as *Ciona* (Morgan, 1904, 1910) have in general agreed in considering that a similarity or lack of differentiation is responsible for the sterility. The writer has already (1916) discussed these conceptions and has presented for consideration the view that the evidence is more readily to be interpreted on the basis of the principle that in general a marked degree of similarity in constitution is necessary for sexual fertility. In this relation it is to be noted that inbreeding in the variety "red-leaved Treviso" has led to a somewhat greater similarity in general characteristics than existed in the original stock grown from commercial seed. In this sense the continued inbreeding of sister plants has led to a greater homozygosity. It is in the 1916 cultures of the offspring of inbred plants that self-fertile plants appeared as noted above. As far as the results in chicory extend, and it may be said that there are no more comprehensive data to be had for any other species, the general results are not in disagreement with the view expressed above.

The sporadic variability of the sex relations and their fluctuating inheritance is very obvious in chicory. Self-fertile plants appear irregularly among the offspring of wide crosses and among plants of inbred strains which are prevailingly self-sterile. In both types of offspring the number of self-fertile plants that appear varies considerably. The manner of their appearance is not to be correlated closely with similarities or dissimilarities as these are ordinarily judged by the expression of characters. The condition of complete functional sex vigor is in many hermaphrodites so complete that it appears to be very definitely fixed in heredity. In chicory, however, we see that highly individual and epigenetic developments may arise, evidently in differentiation and in the transition to the gametophytic stage, which lead to wide and sporadic variations in the functional sex vigor.

The various phenomena of self- and cross-compatibility and incompatibility raise many questions that are fundamental to an understanding of morphogenetic differentiation involved in sexuality, but of which we have at the present time only a superficial knowledge.

When does physiological incompatibility begin to develop? Is it a steady and progressive development through the whole diploid association of the two parental cell elements involved, or is it achieved suddenly at some particular point in ontogeny? Also, when does the sexual condition as distinct from the asexual condition actually arise?

Does incompatibility arise because of sex? Are the two the same? It would seem most definitely that they are not and that incompatibilities are not merely due to sexuality. But even if independent, where incompatibilities do arise, where, how, and to what extent are they correlated with sex and is the development of the two ever parallel? To what extent are the physiological interrelations of sexuality and incompatibility dependent on such mechanical or chemical interactions as are involved in reduction and sporogenesis?

Are the differences of intra-varietal physiological compatibility and incompatibility (both self and cross) indicative of differences in sexuality as such? Are some of the organs of either sex (microgametophytes and macrogametophytes with their respective gametes) sometimes more sexual or of greater sex vigor than are others?

To what degree are the incompatibilities, and compatibilities as well, determined by nutritive relations that are to be considered as vegetative functions? Is sexuality in its origin and in its phenomena of cell fusions, as some have held, to be considered in reality as a phase of vegetative function? To what extent are the sexual incompatibilities related to phenomena of serum incompatibilities and to immunity and what are the fundamental reactions involved in the development and operation of these?

These are among the fundamental questions that naturally arise in connection with such sporadic behavior of functional sex vigor as is seen in chicory in which self-fertile plants of varying degrees of fertility arise among a progeny even after three generations of parentage known to be self-sterile.

New York Botanical Garden

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INHERITANCE OF ENDOSPERM COLOR IN MAIZE[1]

Orland E. White

Few of the many characters in plants and animals studied by geneticists during the last seventeen years are now to be regarded as inherited in simple fashion. As more detailed and extensive studies on the heredity of each type of character, once regarded as a simple unit, are made, the more various the facts and the more complex the interpretations have become. The present paper describes such an increase in complexity of fact and interpretation in the heredity of endosperm color in maize, which at the outset was regarded as a single allelomorphic pair consisting of yellow and white, but which at present involves possibly as many as four pairs of factors, one of which brings about a dominance of white.

As studied and interpreted by Correns (3, 4) and Lock (10) and others, yellow endosperm in maize is determined by the presence of a factor for yellow, in the absence of which, the endosperm remains white. Lock (10) found various degrees of yellow among the grains classified as yellow, but all were easily separated from the white, so that he regarded them as fluctuations of slight importance, and, in his interpretation, did not distinguish between them. He notes, however, that on the average, homozygote yellows are deeper colored than heterozygotes. Lock studied very large numbers of F_2, F_3, and F_4 generation hybrid plants from crosses of yellow and white endosperm varieties. Back-crosses of the yellow heterozygote with the recessive white homozygote were also made in large numbers. The numerical results are very slightly vitiated by the technique used, but the numbers are so large as to make the small error from this source, in this particular case, of comparatively slight importance. Lock's results are summarized in Table 1.

Further studies by East and Hayes (5, 7), Burtt-Davy (1) and others on these endosperm colors brought to light a more complex state of affairs, for they found two yellow endosperm "colors" in maize, each behaving, when crossed with non-yellow (white) endo-

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[1] Brooklyn Botanic Garden Contributions No. 18.

TABLE 1

Cross	Yellow	White	Total	Ratios Act. Obt.	Ratios Theor. Exp.
Yellow heterozygote × white homozygote (F_2)	1,963	1,982	3,945	49.6 :50.4	50:50
Yellow heterozygote × pure white variety	26,792	26,751	53,543	50.03:49.97	50:50
White variety (homozygote) × yellow heterozygote	2,723	2,846	5,569	48 :52	50:50
Totals	31,478	31,579	63,057	49.6 :50.4	50:50
F_2 white segregate × pure white variety	59 all white ears				all
Yellow heterozygote (selfed)	16,592	5,681	22,273	74.5 :25.5	75:25

sperm varieties, as an independent allelomorphic pair. In the varieties studied by East and Hayes, the two yellows were indistinguishable except that ears in which both were present in homozygous condition were usually darker than ears homozygous for either one of the yellows alone. Apparently either of the yellows, even in a homozygous condition, could not be distinguished from the other, but, when both were present in crosses with non-yellow races, the F_2 ratio approximated 15 Y : 1 W. Designating the two factors as Y_1 and Y_2, the presence of either produced a similar effect. In some crosses, dominance of yellow was complete and heterozygotes were indistinguishable, while, in other crosses, as many as five shades of yellow were present among the F_2 progeny, each shade signifying a difference in factorial composition from darker to light in the following order: $Y_1Y_1Y_2Y_2$, $Y_1Y_1y_2y_2$ or $y_1y_1Y_2Y_2$, $Y_1y_1Y_2y_2$, $Y_1y_1Y_2Y_2$ or $Y_1Y_1y_2Y_2$, $Y_1y_1y_2y_2$ or $y_1y_1y_2Y_2$. In Table 17, p. 56 (7) East and Hayes refer to an F_2 population from white × yellow endosperm which gives only a 3Y:1W ratio, but two shades of yellow are distinguishable—a dark and light in the ratio of 1 : 2. This is interpreted as a 1 : 2 : 1 monohybrid ratio in which the heterozygote is easily distinguished, owing to imperfect dominance. The nature of the starch, whether soft (flour) or horny (corneous), also causes a variation in the intensity of the yellow endosperm color. From certain crosses, in a few cases, apparently white segregate seeds, when planted, gave either pure yellow or 3 Y : 1 W progeny.

East and Hayes's data are partially summarized in Table 2:

TABLE 2

Segregation of Endosperm Color in Maize

Cross	Yellow	White	Total	Ratios Act. Obt.	Ratios Theor. Exp.
F_2 generation of white × yellow or reciprocal or data on hybrid population of same character	9,458	466	9,924	95.1: 4.9	93.8:6.2 (15:1)
F_2 generation of white × light yellow or reciprocal or data on hybrid population of same character	6,792	2,428	9,220	73.7:26.3	75:25
F_2 generation of white × dark yellow or reciprocal or data on hybrid population of same character	2,376	766	3,142	75.7:24.3	75:25
F_2 generation of white × yellow	609 dark yellow:1,143 light yellow: 589 white				
Expected	585.2 dark yellow:1,170.4 light yellow: 585.2 white				

The varieties studied by Burtt-Davy (1) apparently consisted of two distinct yellow types—a dark and a light, each of which with its "opposite" represented an independent allelomorphic pair. The darker of these yellows gives a 1 : 2 : 1 ratio, while "the other (the paler) gives the ratio 9 : 3 : 3 : 1." The writer is at a loss to understand the meaning of the above quoted statement, unless the two yellows were crossed together and gave in addition to three types of yellow, whites in the ratio of 1 W : 15 Y. If each yellow is represented by an independent factor, crosses with white should give in each case in F_2 only 1 : 2 : 1 or 3 : 1 ratios. This pale yellow has sometimes been mistaken for a "dominant white." Burtt-Davy found ten shades of yellow in the F_3 seed generation from crossing yellows with whites. Further, Burtt-Davy (p. 172, 173, 177, 188) refers to a yellow endosperm color, which depends for its expression on the presence of two factors—a color factor and a pigment factor. In absence of either, the endosperm will be white, and by crossing two white races, each carrying one of the factors, the resulting progeny will all have yellow endosperm.

Emerson (8) obtained from F_2 populations of crosses of the orange yellow Queen's Golden with Black Mexican (white endosperm), two yellow endosperm colors (a dark and a pale) in addition to the expected orange and white endosperm segregates, and in F_3, some of the pale yellow segregates bred true, while others gave 3 pale yellow : 1 white.

Collins (2) reports a dominant white (albinistic) mutation in endosperm color. The mutation consisted of a single, wholly white ear which appeared in a field of a carefully selected strain of dark yellow dent with red cobs known as Gorham Yellow Dent.

The "albinistic" ear was fully matured, had white cobs, and the seeds when closely examined had a faint trace of yellow at the base, but would ordinarily be regarded as pure white. This white was evidently "dominant" over the Gorham Dent yellow, since the original mutant ear must have been in part, at least, cross-pollinated from the surrounding plants—all of which were Gorham Dent. The immediate descendants of this ear, either when selfed or cross-pollinated, consisted of both yellows and whites, the former greatly predominating. The yellows consisted of both dents and pale types, the former being the more numerous. Seeds of the three color types were grown. From 19 white seeds, 17 all white self-pollinated ears and two white tinged yellow ears were obtained. From 16 light yellow seeds were obtained 2 very light yellow ears, 11 ears with light yellow and white seeds approximating in many cases a 3 Y : 1 W ratio, 1 ear with dark yellow and white seeds approximating a 3 : 1 ratio, and 2 ears unclassified. Forty-nine dark yellow seeds produced 16 ears with yellow of varying shades, 22 ears with both yellow and white grains in the ratio of 3 : 1 (8,694 Y : 2,954 W), 4 ears with both yellow and white grains in the ratio of 15 Y : 1 W (2,548 Y : 177 W—theoretically expected 2,555 Y : 170 W), and seven ears with all shades from dark yellow to white. Crosses between a second generation progeny plant from the albinistic ear (presumably heterozygous for yellow and white) (no. 47) with two white seeded varieties of corn, in both cases gave ears with both white and yellow grains—the ratios approximating 1 : 1. Selfed yellows (44 seeds) from various ears of the first cross (no. 47 × White Dent) gave yellows and whites approximating a ratio of 3 Y : 1 W (44 ears with 16,351 Y : 5,184 W, theoretically expected 16,151 Y : 5,384 W). Selfed yellow seeds from the second cross (no. 47 × white Hopi) gave 2 ears with both yellow and white seeds, the yellow predominating and one "*pure white ear*." Seeds of the selfed pure white ear gave 5 pure white ears and 4 faint yellowish tinted white seeded ears. No data are given as to cob color in the case of the second appearance of a white endosperm mutation, nor in the case of the dozen or more pure white ears obtained in later generations. Since both the white cob and white endosperm appeared together as a single mutation, it would be interesting to know whether they were inherited together, and whether the white cob color was also dominant over the red from which it sprung. Owing to ratio discrepancies, and the occurrence of traces of yellow in descendants of seeds classed as *pure* white, Collins regards the segregation of yellow and white endosperm color as incomplete or imperfect. In other words, factor contamination has occurred, though, in general, Mendelian ratios were obtained. Interpreting his results as showing the presence of at least two factors for yellow color and perhaps more, Collins regards as both "violent" and unwarranted. East (6, p. 404–405) however, in reviewing this paper, interprets Collin's data as demonstrating the presence of two factors for yellow endosperm color, one of which is much less effective in producing the yellow color than the other. East discredits the mutative reversal of dominance interpretation, suggesting the appearance of the original wholly white ear as due to non-development of color brought about by abnormal environmental causes, such as, perhaps, the "accidental presence" of some metallic salt in the soil. This suppression of color development, East intimates, is not extremely rare in experimental corn cultures.

New Data

The material consisted of an inbred strain of California Golden Pop with yellow endosperm and a strain of white endosperm maize obtained from Haage & Schmidt under the name of *Zea Caragua*.[2] The latter bred true to a white endosperm intermediate between flour and corneous in texture. A white endosperm variety of Hopi maize, isolated from seed obtained from G. N. Collins, was also used.

In classifying the colors of the F_2 and F_3 seeds, three methods were used—(1) each seed as classified on the ear, was picked off and transferred to a black velvet background and contrasted either with the parental varieties or with other pure white and yellow races; (2) most of the seeds were classified independently by the writer and his assistant Miss M. Mann; (3) both the writer and Miss Mann reclassified many of the F_2 and F_3 ears twice, first by re-examining both the yellow and white groupings of each ear and second by mixing the two color groups together again and reselecting. In each reclassification, the ratios resulting from previous classifications were unknown. These determinations were fairly accurate as demonstrated by the F_2 classification and its F_3 progeny. In some cases a photographic blue ray screen was used.

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[2] Described by Sturtevant (11). Apparently an old variety desseminated in Europe by Vilmorin.

F_1 GENERATION PROGENY

California Golden Pop pollinated by *Zea Caragua* gave uniformly white endosperm grains with perhaps the very faintest suggestion of yellow.[3] This cross was repeated six times with similar results. The reciprocal cross has not yet been obtained. Crosses of Golden Pop pollinated with white endosperm Hopi gave similar results.

F_2 GENERATION

Three independent classifications of the F_2 progeny from California Golden Pop × *Zea Caragua* are given in Table 3. The first three ears were classified with the aid of a photographic blue ray screen.

TABLE 3

Endosperm Color of F_2 Progeny of (Z 14×Z 21)

Progeny No.	1st Classif.		2d Classif.		3d Classif.		Total Grains
	White	Yellow	White	Yellow	White	Yellow	
(Z 14×Z 21)—1	254	82	Practically		—	—	336
(“ “)—2	264	108	the same		—	—	372
(“ “)—3	285	89	“	“	—	—	374
(“ “)—4[4]	257	79	235	101	225	111	336
(“ “)—5[4]	346	73	319	100	267	152	419
(“ “)—6[4]	338	98	358	79	340	96	436
(“ “)—7[4]	346	77	316	109	259	166	425
(“ “)—8[4]	248	70	235	82	214	104	318
(“ “)—25*a*	350	146	311	185	354	142	496
(“ “)—25*b*	358	129	350	137	339	148	487
(“ “)—26*a*	422	126	420	128	410	138	548
(“ “)—26*b*[5]	494	109	483	121	472	132	604
(“ “)—28	336	91	318	109	315	112	427
(“ “)—29*b*	478	131	460	149	438	171	609
(“ “)—30	390	143	406	127	388	145	533
(“ “)—31	373	54	313	114	316	111	427
(“ “)—32	332	104	332	104	331	105	436
(“ “)—33	427	73	371	129	386	114	500
(“ “)—34*a*	221	52	209	64	211	62	273
(“ “)—35	309	93	295	107	280	122	402
(“ “)—36	375	95	379	91	369	101	470
(“ “)—37	338	97	328	107	282	153	435

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[3] The F_1 white endosperm of these seeds is indistinguishable from that of seeds of several of the well known smooth white seeded varieties of pop-corn, when compared with them. From data on non-guarded crosses referred to later, the reciprocal cross probably gives the same results, except the endosperm is opaque white instead of translucent white.

[4] Ears obtained from unbagged F_1 plants allowed to intercross with other F_1s of similar pedigree.

[5] One unclassified.

Owing to the increased experience, the third classification given in Table 3 is probably the most accurate. Assuming that it is, from a total of 9,663 progeny, 6,999 were classed as white and 2,664 as yellow. On the assumption of a one-factor difference between the two maize races with complete or practically complete dominance of the white color, the theoretically expected numbers would be 7,248 W : 2,416 Y (3 : 1). The deviation between the ratio actually obtained and that theoretically expected is 249. The yellow segregates were far from uniform in color, all shades from a dark yellow (not orange) to a very light lemon yellow were present on the same ear. Further, in some ears, the yellow was principally confined to the base of the grain, nearest the point of attachment. A few dark yellow grains were somewhat deeper colored than the yellow grand-parental California Pop, but this may be due to segregation of various factors that effect endosperm texture, as the dark yellow grains usually appear less translucent than those of the grand-parental Pop variety.

BACK CROSSES OF F_1 WITH Z 21 (THE DOMINANT WHITE ENDOSPERM PARENT)

(Z 14 × Z 21)–29a × Z 21 . . . . . . All white
(Z 14 × Z 21)–34b × Z 21 . . . . . . All white

These two ears, resulting from back crosses of the F_1 with the dominant endosperm parent, came from two F_1 plants which also produced two selfed ears (29*b* and 34*a* in Table 3). The back-crossed ears were uniform in seed color, the white being more opaque than in the endosperm of the F_1 grains. Nos. 29*b* and 34*a* gave typical F_2 ratios and the yellows were of several shades as in all the other ears with F_2 seeds.

F_3 GENERATION

From self-pollinated ears Nos. 1, 2 and 3 (Table 3) of the F_2 generation, approximately 1,000 plants with F_3 endosperm seeds were grown. Of these, 43 ears were self-pollinated, 27 of which came from F_2 seeds classed as white, and 16 from F_2 seeds classed as yellow. Nine of the white seeds gave all white F_3 progeny, while 19 gave both white and yellow grains approximating the ratio of 3 W : 1 Y (Table 4). The 16 F_2 seeds classed as yellows gave 14 all yellow ears, and 3 ears with both white and yellow seeds approximating the ratio of 3 W : 1 Y.

TABLE 4

F_3 Progeny of White-Seeded F_2 Heterozygotes (*Z* 14×*Z* 21)

Plant No.	White	Yellow	Total No Grains
1 W—63	269	55	324
1 W—89	294	98	392
1 W—90	119	38	157
1 W—91*a*	277	122	399
1 W—93	364	88	452
1 W—98	233	74	307
1 W—99	298	108	406
1 W—101	298	140	438
2 W—66	309	75	384
3 W—43*a*	113	48	161
3 W—43*b*	190	90	280
3 W—48	143	70	213
3 W—49	119	39	158
3 W—52	343	104	447
3 W—53	216	56	272
3 W—56	59	22	81
3 W—57	417	72	489
2 Y—77*a*[6]	212	78	290
2 Y—77*b*[6]	245	81	326
2 Y—84[6]	185	47	232
Total actually obtained	4,703	1,505	6,208
Total theoretically expected	4,656	1,552	6,208

Both yellow and white endosperm colors varied markedly in this generation. In the case of white endosperm, the differences were largely due to segregation of factors affecting the texture and degree of translucency and opaqueness. Many ears had opaque caps, while the remainder of the endosperm was corneous. In such cases, the yellow was most apparent in the corneous region. Translucent whites such as one finds among popcorn varieties always appear slightly yellow when contrasted with opaque whites such as are found among the dent and wax varieties. No selfed ears were obtained of a deeper yellow color than that in the California Pop ancestor. The all yellow ears were of at least three distinguishable types: (1) a very light translucent lemon yellow, (2) a yellow as dark as the ancestral yellow and (3) a yellow with opaque whitish caps.

UNBAGGED EARS OF Z 14 AND Z 21

Unbagged ears on plants of Z 14 grown close to varieties with deep yellow or orange endosperm color invariably have a large number of dark yellow or orange grains, from which the dominance of these yellows over that of Z 14 (California Pop) is to be inferred, as bagged ears are always of a uniform medium yellow.

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[6] F_2 seeds probably wrongly classed as having yellow endosperm.

Unbagged ears of Z 21 (*Zea Caragua*) on plants grown under conditions similar to those mentioned for Z 14 have never been found in my cultures with yellow grains. Further, cultures of Z 21 grown alongside of F_1 and F_2 generation hybrids (Z 14 × Z 21) have always produced (in my experience) only white ears. The Z 21 cultures bloomed at about the same time as many of the hybrids, so that the difference in flowering time would not account for the absence of yellow grains.

INTERPRETATION

In the light of the preceding data, endosperm color differences between Z 14 (California Pop) and Z 21 (*Z. Caragua*) may be regarded as due to the presence and absence of a single factor A. The presence of A prevents the development of the yellow color, when the factors for yellow pigment are present, and gives no indication of its presence in a variety from which these factors are absent. In the absence of A, a given variety may be either yellow or white. In respect to this factor A, then, and a single factor for yellow pigment, varieties of corn may be of four kinds:

(1) AAYY (white endosperm)
(2) AAyy (white ")
(3) aaYY (yellow ")
(4) aayy (white ")

Crossed with each other, these should give:

Cross	F_1	F_2
1(W)×2(W)	white (AAYy)	all white
1(W)×3(Y)	white (AaYY)	3 W:1 Y
1(W)×4(W)	white (AaYy)	13 W:3 Y
2(W)×3(Y)	white (AaYy)	13 W:3 Y
2(W)×4(W)	white (Aayy)	all white
3(Y)×4(W)	yellow (aaYy)	3 Y:1 W

So far as the data on Z 14 × Z 21 are concerned, California Golden Pop would be represented on the above scheme as aaYY, while the formula AAYY would be the only one applicable to Z 21 (*Z. Caragua*). All of the common white endosperm varieties of corn which are wholly or partially recessive to yellow endosperm color have the formula aayy, neither the suppression nor the pigment factor being present.

First generation (F_1) progeny from crosses between whites such as 1 × 4, or whites and yellows such as 2 × 3, should give, when backcrossed with white endosperm aayy (4) plants, white and yellow endosperm seeds in the ratio of 3 W : 1 Y. In other words, two whites crossed together in F_1 give a certain proportion of yellows. The obtaining of such results in partially worked out experiments on heredity of endosperm color, in which it was taken for granted that white endosperm color was always recessive, might be temporarily interpreted as due to the presence of a color factor in a heterozygous condition in one of the races experimented with. Perhaps Burtt-Davy's statements regarding the presence of a color factor for endosperm (1, pp. 172, 173, 177) resulted from an experiment of this type. I have not had access to papers with the data on which these statements are based.

The preceding discussion assumes only one factor for yellow endosperm pigment, whereas East and Hayes, Collins and Burtt-Davy have each found at least two such factors. Further, Emerson and East (9, p. 11) suggest that orange endosperm color, such as is characteristic of Queen's Golden Pop, Tom Thumb Pop, Yardstick and some Chinese varieties (much intensified), is due possibly to the presence of a color intensifying factor. The F_2 and F_3 data on crosses of Z 14 × Z 21 show the presence of other color modifying factors, especially one which dilutes ordinary yellow to a very pale lemon color. Other investigators have also obtained this type.

Summary

1. Crosses of a yellow endosperm variety of maize (California Golden Pop) with a white endosperm variety (*Z. Caragua*) gave uniformly white progeny in F_1 and a ratio approximating 3 W : 1 Y in F_2. The F_2 generation white grains, when planted, gave either all white F_3 generation progeny or a mixture of white and yellow grains approximating a ratio of 3 W : 1 Y. The F_2 yellow grains, except in two cases, produced all yellow F_3 self-pollinated ears. The yellow grains in both the F_2 and F_3 generations varied considerably, and, in F_3, ears wholly of *very* light lemon yellow grains were obtained. Unprotected ears of Z 21 in close proximity to varieties and hybrids having yellow endosperm always gave wholly white endosperm ears.

2. These results are interpreted as mainly due to the presence and absence of an endosperm color suppression factor A. A factor Y for yellow pigment is present in both races studied. *Zea Caragua* (Z 21) is to be regarded as homozygous for both A and Y, while California Golden Pop (Z 14) is homozygous for the presence of Y and the absence of A.

3. The segregation of other endosperm factors, such as those for flint and floury texture, opaque caps, etc., also modified the endosperm color expressions.

4. Including the suppression factor A, at least three and possibly five pairs of factors are primarily responsible for endosperm color in maize.

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THE INFLUENCE OF LIGHT AND CHLOROPHYLL FORMATION ON THE MINIMUM TOXIC CONCENTRATION OF MAGNESIUM NITRATE FOR THE SQUASH*

R. B. Harvey and R. H. True

In testing the absorption of magnesium nitrate by the squash (Early Prolific Marrow), varying results in different series led the authors to investigate the causes of these differences.

The results presented are taken from four experiments, the first of which was run in full sunlight under a glass cover in the greenhouse at a temperature varying between 17° and 30° C. This series (see graph) showed a concentration of $200n \times 10{-}6$ $Mg(NO_3)_2$ to be toxic to the squash while $120n \times 10{-}6$ was not toxic. A series run in the dark at a temperature of 18° showed both the preceding concentrations to be toxic.

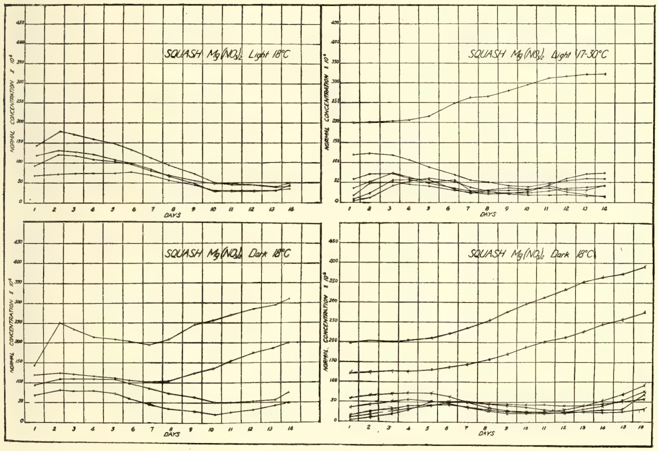

FIG. 1. For explanation see text.

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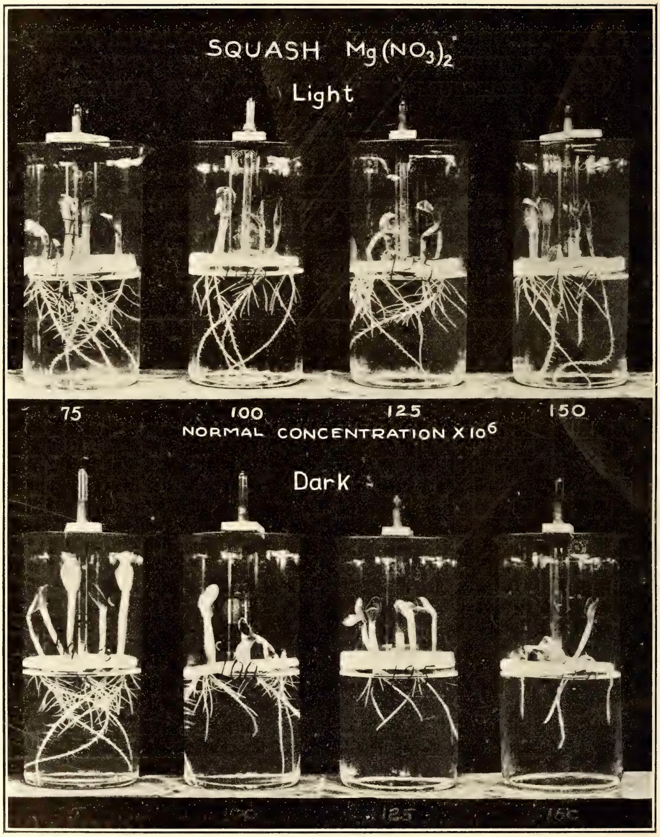
SQUASH Mg(NO3)2
Light
75
100
125
150
NORMAL CONCENTRATION X 10^6
Dark
75
100
125
150

Fig. 2. For explanation see text.

To eliminate the effect of temperature, the above series were repeated, both at a constant temperature of 18° C. Concentrations of exactly the same value were used in each. In the series exposed to the light, a diffused daylight intensified by a tungsten lamp was used. Heat radiation from the lamp was minimized by a double glass window in the constant temperature room. In every plant of this series there was a deep green coloration equal to that of plants grown in daylight. As is shown by the graph, a concentration of 150*n* × 10–6 $Mg(NO_3)_2$ is not toxic to the squash under the above conditions of illumination. In the dark, however, this concentration is toxic. The toxicity here is plainly shown both by the leach of electrolytes with an increase in concentration as shown by the graph, and in the lack of root growth as shown by the photograph. The growth of tops in the etiolated seedlings is of course greater than those exposed to light. The concentration 125*n* × 10–6 seems to be just at the border line of toxicity for etiolated squashes. It, therefore, appears that such light exposure and chlorophyll formation is accompanied by a rise in the minimal toxic concentration of the solution.

Either of two conditions can produce this rise in the minimal toxic concentration. Either the resistance of the protoplasm to the toxic effect of magnesium may be increased, or the concentration of magnesium within the cell may be reduced by light exposure. In regard to increased resistance of the protoplasm no evidence is offered here. It seems probable, however, that the decrease in concentration brought about by light exposure may be sufficient to account for the change. To become toxic the magnesium must reach a certain concentration within the cell. From the etiolated series this minimum toxic concentration is seen to be a little below the equilibrium concentration established within the cells in 120*n* × 10–6 $Mg(NO_3)_2$. Since a concentration of 150*n* × 10–6 $Mg(NO_3)_2$ is not toxic to plants exposed to light, the concentration of $Mg(NO_3)_2$ actually in condition to produce toxic effects within the cell is probably less than the minimum toxic concentration found for etiolated seedlings. This decrease in concentration may be brought about by the removal of magnesium to form non-toxic compounds. One such group of compounds comparatively rich in magnesium whose formation in the squash depends upon light is the leaf-green compounds found by Willstätter (1) to consist of two parts, chlorophyll *a* and chlorophyll *b*.

From the work of Willstätter and others (2), it has been shown that magnesium forms an important part of the chlorophyll molecule. Mameli (3, 4), has shown that the presence of magnesium favors chlorophyll formation.

By means of the Grignard reagent Willstätter and Stoll were able to introduce magnesium into the substance aetioporphyrin $C_{31}H_{36}N_4$ to form aetiophyllin $C_{31}H_{34}N_4Mg$, one of the cleavage products of the chlorophyll molecule. The results of experiments here presented seem to indicate that the introduction of magnesium into the compounds of the leaf takes place to a greater degree when there is sufficient illumination to cause a green coloration, that is, the squash requires light for the later steps of chlorophyll synthesis and these steps are associated with the removal of magnesium from the field of toxic action. No quantitative measure of the amount of chlorophyll compounds present has been obtained on account of their instability and complexity. However, calculations using the formulae found by Willstätter indicate that the increased amount of magnesium used in the light is well within the limits of the amount used for chlorophyll synthesis as determined by Willstätter in nettle leaves.

In testing the toxicity of ferric chloride solutions under similar conditions, no differences were observed between cultures grown in the light and darkness.

SUMMARY

The minimal toxic concentration of magnesium nitrate for the squash grown in water cultures was found to be $125n \times 10^{-6}$ in the dark and $200n \times 10^{-6}$ in the light. The increase in the minimal toxic concentration is probably correlated with the removal of magnesium from toxic compounds to form chlorophyll.

Bureau of Plant Industry,
U. S. Department of Agriculture.

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THE USE OF THE VIBRATION GALVANOMETER WITH A 60-CYCLE ALTERNATING CURRENT IN THE MEASUREMENT OF THE CONDUCTIVITY OF ELECTROLYTES

Newton B. Green

In 1898 Kohlrausch (1) published a description of his method for determining the conductivity of electrolytes, and since that time much has been done by various investigators to increase the accuracy of the method. Notable among these are E. W. Washburn (2) and R. P. Hibbard in collaboration with C. W. Chapman (3). Still more recently has appeared an article by W. Taylor and S. F. Acree (5). Among the sources of error, which they have removed, may be mentioned the following: an alternating current from an induction coil which is neither strictly alternating nor of constant frequency; resistance coils which are inaccurate because of capacity and inductance; and lack of sensitivity in the telephone detector. At the present writing a series of articles is appearing in the Journal of the American Chemical Society by Dr. Washburn (4) which sums up the latest researches on the subject. To these articles any investigators who desire *absolute* accuracy of results are referred. The plant physiologist is concerned more with precise comparative data than with absolute physical accuracy, which must of necessity include experiments extending over long periods of time and involving great elaboration of method.

Dr. Washburn's method overcomes the difficulties mentioned above in the following manner: He uses for a source of current either the Vreeland Oscillator, which gives a pure sine wave at a frequency of one thousand cycles per second, or a constant-speed high-frequency generator which delivers an alternating current at the same frequency. Both of these pieces of apparatus and the principles involved are described in Catalog No. 48 of the Leeds and Northrup Co. (6). For resistance coils he uses the Curtis type, which have a minimum of inductance and capacity, and for the detector he uses a telephone receiver tuned to the frequency of the current. He also finds it necessary in connection with the high frequency generator to maintain the correct resonance in the bridge circuit by means of a double condenser. Consequently a complete outfit for making conductivity measurements, using the generator and excluding bridge, conductivity cell and resistance coils, which are of course necessary in any form of apparatus, would cost over two hundred and fifty dollars. If the oscillator is substituted as a source of current the price is increased to about three hundred dollars. In either case a condenser must be used to balance out the capacity in the conductivity cell.

Hibbard and Chapman have met the problems as to source of current and detector in a different manner. They use a 60-cycle rotary converter and an alternating current galvanometer of the electro-dynamometer type. The latter costs about one hundred dollars. In addition, a rheostat is necessary to regulate the primary current. Consequently this apparatus exclusive of bridge, cell and resistances must cost as much or more than that employed by Washburn, without being applicable to as wide a variety of conditions. The physical chemist will occasionally need a current of higher frequency than 60 cycles, and such variation is impossible with the ordinary rotary converter. Obviously the main objection to both of these methods from the standpoint of the plant physiologist is the one of expense involved. This is especially significant when one considers that the apparatus employed serves one purpose only in the plant physiology laboratory, namely, that of measuring changes in permeability, or the electrolytic content of plant tissues and juices. Consequently the writer considers it likely that an apparatus embodying the latest methods of procedure, which fulfills all the requirements of precision, will be welcomed by workers along this line.

Since the investigators, whose results are cited above, have conducted the most exhaustive researches on the subject of conductivity measurements, it is certainly desirable to follow any procedure which they all recommend. We may assume then the necessity for a constant temperature bath, in which to immerse the conductivity cell, and a condenser to balance out the capacity in the cell. It is also certain that the Curtis coils are the most reliable of all available resistances, because they are so wound as to reduce inductance and capacity to a minimum. By standardizing the apparatus to this extent we are sure that the results obtained will have at least precise comparative values. We now come to the question of the source and type of current to be used. Washburn is of the opinion that to avoid undue polarization in the conductivity cell, a frequency of 1,000 cycles per second is necessary. Moreover Taylor and Acree have shown that as the frequency approaches infinity, variations in the resistance and capacity of the cell approach zero. If we adopt this high frequency, the only available type of detector is the telephone receiver. On the other hand, Hibbard and Chapman, after exhaustive experimentation with lower frequencies, assert that at 60 cycles per second, using a cell with platinized electrodes, the amount of polarization is practically negligible in all but a few exceptional types of solutions. If this is the case the plant physiologist may feel secure in using this frequency, which has several great advantages over higher frequencies as will now be explained. An additional security rests in the fact that polarization, if present, is easily detected in the "creeping" of the balance point, and can be immediately remedied by cutting down the amount of current and the period of time in which the circuit is closed. The main advantage in using a low frequency lies in the fact that another detector than the telephone may be used. Such a detector is the alternating current galvanometer, of which there are two general types. The advantages of such a substitution are many and are fully discussed by Hibbard and Chapman. All who have worked with the telephone as detector will understand the difficulties attending the constant strain of listening, and will appreciate the substitution of a method which enables sight to take the place of hearing.

At this point the alterations in apparatus devised by the writer may properly be considered. If a frequency of 60 cycles per second is possible without a sacrifice in precision of comparative results, there should be some source of current more available and entailing less initial cost than the rotary converter. Such a source of current is present in practically every laboratory, and needs only to be reduced to the proper E.M.F. and potential. This is the ordinary 110-volt alternating-current lighting circuit. As supplied to the laboratory it is practically always a single-phase, 60-cycle system, having in most cases a frequency variation of not more than one percent and a remarkably pure wave form. Taylor and Acree in their article have inserted oscillograms of the Madison (Wis.) city current, which are by no means exceptional, and can be duplicated elsewhere. For example when this type of current is used to supply a bridge network in which the bridge-wire has a resistance of 1.2 ohms, the current can be sufficiently reduced by the insertion in series of two 16 c.p. lamps. When the connections are made as in the diagram, the current passing through the bridge wire, if R and R' are open, is about .28 amperes at a potential of .336 volt.[1] In practice the resistances R and R' are in series with each other and the two connected in parallel with the bridge-wire. Then when the bridge is balanced, the current divides itself between $R + R'$ and the bridge-wire, so that the two divisions of the current are in inverse proportion to the resistances of these parallel branches. Since the resistance of the bridge-wire is in this case 1.2 ohms and the resistance of the unknown (R') + its balance resistance (R) varies between 200 ohms for concentrated solutions and 10^6 ohms for conductivity water, it is easily seen that the amount of current passing through R' will be exceedingly small in all cases, running from .0016 ampere to 3.36×10^{-7} amp. If however heating occurs when a concentrated solution is being measured, it can easily be obviated by the introduction of a rheostat in series with the bridge-wire and lamps thus cutting the current down still further.

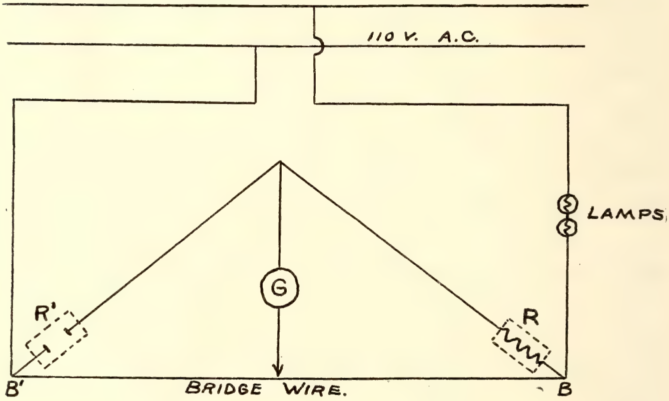

FIG. 1. For explanation see text.

There now remains the consideration of the detector. For frequencies around 60 cycles the vibration galvanometer, as made by Leeds and Northrup for seventy-five dollars, is the most sensitive. It is easily tuned to the exact frequency of the current supply and once tuned needs attention only on rare occasions. Its sensitivity is such as to make profitable the use of the most accurate bridge with extensions on the bridge-wire. An added advantage is that the moving coil returns quickly to its neutral position when the circuit is broken, so that the band of light from the mirror follows closely in its width the position of the slider on the wire. This enables a speedy determination of the balance point and cuts down the chance of polarization.

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[1] The writer is indebted to Dr. Alan T. Waterman, of the Department of Physics of the University of Cincinnati, for assistance in calculating the electrical data.

The total cost of such an apparatus using the best bridge, resistances, conductivity cell and condensers will be much less than either the Washburn or the Hibbard and Chapman outfits. This is made possible by the substitution of the city current for an expensive piece of apparatus, which is itself often a source of annoyance because of noise. Moreover the vibration galvanometer is less expensive than the electro-dynamometer type, and there is no sacrifice in precision. The writer believes that these advantages will appear to be of distinct importance to plant physiologists and to others interested in conductivity measurements.

SUMMARY

Since Messrs. Hibbard and Chapman have shown that polarization is in nearly all cases a negligible factor using a current of 60-cycle frequency, the ordinary single-phase, 110-volt, a.-c. lighting circuit can be used as a source of current in making measurements of the conductivity of electrolytes.

With such a frequency the most sensitive and convenient detector is the vibration galvanometer.

The use of this method in preference to those previously known enables the investigator, who desires only precise comparative results, to make a considerable saving in first cost of apparatus without any attendant sacrifice in accuracy.

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University of Cincinnati,
Department of Botany.

IMMUNOCHEMICAL STUDIES OF THE PLANT PROTEINS: PROTEINS OF THE WHEAT SEED AND OTHER CEREALS. STUDY IX[1]

R. P. Wodehouse

That wheat foods are active in causing asthma has become an established fact and it has been shown that watery extracts made from practically any of the wheat foods except those cooked at very high temperatures (Goodale '16) will give positive skin reactions when tested by means of the skin test (which is generally considered a test for anaphylactic sensitization) to wheat asthmatics. This present work was undertaken in order to find out which protein or proteins of the wheat seed were responsible for the production of asthma by isolating them individually in as pure a form as possible and testing by means of the skin reaction.

The reserve proteins of wheat have been thoroughly studied by Osborne and his co-workers (Osborne '10, '10*A*, '07). By these investigators many different protein preparations were made from the wheat seed and it was shown by very careful analyses of these that there are five and probably only five distinct reserve proteins present in the seed and these fall into the well-known protein classes as follows:

Albumin of wheat = leucosin
Globulin of wheat = wheat globulin
Prolamine of wheat = gliadin
Glutelin of wheat = glutenin
$\text{Proteose of wheat} = \begin{cases} \text{wheat natural proteose} \\ \text{wheat artificial proteose} \end{cases}$

These proteins can be distinguished by their elementary composition and by their amino-acid content which these investigators have worked out (Osborne '10, Osborne and Clapp '06) but they are most readily distinguished by their solubility characteristics which are used to place them in the groups of plant proteins to which they belong. For convenience they are briefly here summarized as follows:

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[1] Made possible through a gift by Mr. Charles F. Choat, Jr., Boston, to the Peter Bent Brigham Hospital for the study of bronchial asthma.

Leucosin is soluble in pure water or in water faintly acid or alkaline in reaction but is precipitated in an insoluble coagulum from a faintly acid solution by heat.

Wheat Globulin. In neutral media this is soluble only in saline solutions, the one generally used being 10 percent NaCl.

Gliadin is soluble in 50–80 percent alcohol but insoluble in water or absolute alcohol.

Glutenin is insoluble in water, alcohol or neutral salt solution but readily soluble in weak alkali, 1/5 percent being generally used.

Proteose is soluble in water and not precipitated by heat.

All of the proteins soluble in water or salt solutions may be precipitated by saturating their solutions with ammonium sulphate.

Whether these proteins are chemical individuals or whether we have to seek further separations is not definitely known. None has been prepared, as yet, in a crystalline form. Though some vegetable proteins have been crystallized, wheat globulin which, of the wheat proteins, approaches nearest to this condition, occurs only in more or less regular spheroids.

There is pretty good evidence to show that all of these proteins, including the "*natural proteose*," exist preformed in the seed. Proteoses are generally regarded, however, as being the first product of hydrolysis of the higher proteins. Still Wells and Osborne (Wells and Osborne, '15) furnish good evidence that the "*natural proteose*" is not a product of hydrolysis but is a naturally existing protein of the seed.

The *artificial proteose* was prepared by hydrolysis from glutenin (as will be shown) and was used throughout the wheat experiments for comparison with the natural proteins in general and more especially to see if a proteose, known to be the product of hydrolysis, would give biological reactions similar to those of natural proteose.

Besides these individual proteins there were used in this work two "*whole wheat*" protein preparations made, one from raw wheat and the other from bread, by the author (Wodehouse, '16). Of course these two preparations do not, as their name might imply, contain all five of the wheat proteins in anything like equal proportions. On the contrary the solubilities, as indicated above, would prevent such preparations from containing more than a trace of glutenin and gliadin, and the bulk would be made up of the natural and decomposition proteoses together with leucosin and globulin where they were not precipitated by heat.

Method of Preparation

Proteins Soluble in Aqueous Salt Solution

Since these proteins occur mainly in the embryo of the wheat grain and not very much in the endosperm (Osborne and Campbell, '00), ordinary white bread or pastry flour should not be used. The commercial "entire wheat" preparations give very good results except that the yield is small.

Leucosin, being soluble in pure water, can be extracted from the flour by simply soaking in cold water (with the addition of some preservative as thymol or toluol to prevent bacterial decomposition) for a few days, and decanting the supernatant fluid which then contains this protein together with some globulin, dissolved by virtue of the mineral salts contained in the grain, and proteose, together with sugars, etc. This method of preparing leucosin was discarded early in the work because it was found much easier to extract it together with the globulin and separate them afterwards. So the entire wheat flour was stirred in 10 percent salt solution (about 1,200 gm. to 3,500 cc.) and allowed to stand at room temperature for about three days (no preservative is necessary). The flour settles to the bottom and the supernatant solution (which is pinkish and syrupy) can be siphoned off. It is desirable to allow the flour to separate completely from the supernatant fluid so that further clearing will be unnecessary, for the viscosity of this solution renders it difficult to filter. Less than one half of the volume of the salt solution is recovered, the rest remaining entangled in the flour, so it is profitable to make a second extraction from the same flour by adding a volume of salt solution equal to that removed by decantation. This second extraction is almost as rich in protein as the first. Since the globulin is insoluble in water at neutral reaction it may now be separated out by dialyzing the whole solution in water until free from Cl (the other proteins may be separated from each other as will be subsequently shown), or all the proteins may be salted out together by saturation of the extract with ammonium sulphate, and separation effected by dialysis after again being dissolved.

In the preparation of natural proteose it was found desirable to follow the former method. When the salt extract is freed from NaCl by dialysis all of the globulin and possibly parts of some of the other proteins are thrown out of solution and can be removed by filtration.

If the filtrate be faintly acidulated and boiled the leucosin is coagulated and forms a flocculent precipitate which can be removed by filtration. The proteose, which now remains in solution, can best be obtained by reducing the volume by boiling on the water bath and then dialyzing against 95 percent alcohol when it appears in the form of a white precipitate which can be washed in alcohol and ether and dried over sulphuric acid giving a white powder. When prepared in this manner it is perfectly soluble in water or 0.01 M KOH, giving a clear solution.

The globulin precipitated by dialysis from 10 percent NaCl solution is largely insoluble when treated a second time with 10 percent NaCl. For this reason this method cannot be used advantageously for the preparation of globulin. It was found best to follow the method of first saturating the 10 percent NaCl extract with ammonium sulphate thereby throwing out of solution all of the proteins together. This precipitate can then be dissolved in 5 percent NaCl and the solution dialyzed free from Cl and SO_4 when the globulin is thrown out of solution in spheroids or imperfect crystals which can be separated from the solution by centrifugalizing. Globulin thus prepared can nearly all be redissolved in 5 percent NaCl and precipitated again by dialysis. In making the globulin preparations used in this work this was done twice in order to purify the preparations. It was then washed in water, 95 percent alcohol, absolute alcohol and ether and dried over sulphuric acid under diminished pressure. This preparation is completely soluble in 10 percent NaCl or weak alkali. It appears in more or less regular spheroids or imperfect crystals, so it can be considered to be reasonably pure.

Leucosin is very difficult to separate from proteose without coagulation, therefore no attempt was made to prepare it entirely free from proteose. The leucosin used in these experiments was prepared as follows: The NaCl extract, after the salt and globulin had been removed by dialysis was saturated with ammonium sulphate and the proteins thereby precipitated were filtered out and pressed as dry as possible between filter paper and redissolved in a small amount of water, in which they proved to be almost completely soluble. This solution was then dialyzed until free from the remaining ammonium sulphate, or until it failed to give a precipitate with barium chloride. This caused the production of a very small amount of an insoluble *protean* which was filtered out. The solution was then dialyzed against 95 percent alcohol until further reduced in volume. This caused the production of only a small precipitate, so the whole was poured into three volumes of a mixture of acetone and ether (80–20) and the precipitate so formed was centrifugalized out and washed in alcohol and ether and dried over sulphuric acid under diminished pressure. When desiccation was complete it formed a gray powder which was more or less insoluble according to the time of exposure to the alcohol baths.

Proteins Insoluble in Water or Neutral Aqueous Saline Solutions

Gliadin and glutenin occur mostly in the endosperm of the seed and are the proteins which make up *gluten*, the substance which gives what flour its capacity for making dough. In order to obtain the gluten the flour is mixed with water and kneaded into a stiff dough. This is then wrapped in muslin and kneaded under water until the starch is washed out. When it is mostly removed the dough may be taken from the muslin and the kneading continued under water until no further starch can be removed. In preparing the proteins for this work the gluten was next kneaded in several baths of 10 percent NaCl, then chopped fine and allowed to remain in a large volume of salt solution over night to complete the removal of the globulin, then (still chopped fine) it was allowed to remain in running water for some hours to remove the salt.

In order to dissolve out the gliadin it was now subjected to extraction with alcohol. This was done by boiling in 70 percent alcohol on the hot water bath for about an hour, using a reflux condenser to keep the alcohol from evaporating. It was then strained off through muslin and the extraction of the undissolved gluten was continued with a fresh bath of alcohol. In the meantime the first extract was filtered and the alcohol distilled off by heating in a retort on a boiling water bath. The alcohol recovered from this distillation was then diluted with water to make again 70 percent by the hydrometer test, and used for the next bath. This process was repeated five or six times or until nearly all the alcohol soluble protein was removed from the gluten. Care was taken during the evaporation of the gliadin solution in the still not to let too much alcohol evaporate. No attempts were made to see what percentage of alcohol remained or at what temperature it was boiling. Distillation was discontinued, however, while there was still enough alcohol left to lower the boiling point of the solution sufficiently to cause it to boil vigorously on the hot water bath. In this way the risk of heating it to too high a temperature was avoided.

When this gliadin solution was allowed to cool a small part of the protein settled out in a gluey mass at the bottom, and part assumed the form of a fine suspension which would pass through filter paper and could not be removed by centrifugalizing. So it was warmed up enough to cause complete resolution and while still hot poured into the dialyzers and dialyzed against tap water for three days, using thymol as a preservative. At the end of this time the protein had all settled at the bottom. The supernatant fluid was discarded, the dialyzers torn open and the gliadin scraped off. At this stage the protein was light gray in color and resembled malleable rubber in consistency. It was thoroughly washed in distilled water, then cut up into fine pieces and digested successively with acetone and ether, absolute alcohol, ether, and dried over sulphuric acid under diminished pressure. When desiccation was complete it was ground in a mortar to a fine gray powder which could be used conveniently for making the tests in these experiments.

The residue from the gluten, remaining after the extraction of the gliadin, was now soaked in five or six volumes of 0.2 percent KOH to dissolve out the glutenin. This solution formed a thick opaque white fluid which could not be filtered; it was centrifugalized at high speed for about one hour; this precipitated a considerable amount of insoluble material. The supernatant fluid was poured off and very carefully neutralized by adding 1 percent HCl. This caused the production of a voluminous curdy precipitate which reached a maximum at neutrality to litmus and would readily redissolve if made slightly more acid. This precipitated glutenin was removed by centrifugalization and the supernatant fluid, which was found to contain a large amount of protein, was evaporated down to about one fifth of its original volume on the water bath; the small amount of precipitate which had formed was discarded and the solution dialyzed free from KCl. Since no further precipitate was formed the dialyzer was transferred to 95 percent alcohol which reduced the volume still further and caused the appearance of a precipitate. Dialysis was continued in fresh baths of alcohol and finally absolute alcohol and when thus dehydrated the precipitate was removed, washed in absolute alcohol, ether and dried over sulphuric acid under diminished pressure. This gave a fine white powder which is called in these experiments "*artificial proteose*."

The precipitated glutenin was now dissolved in 0.2 percent KOH and centrifugalized to remove a small part that would not dissolve. It was then precipitated again by neutralization with HCl, washed in water, several baths of 70 percent alcohol to remove all traces of gliadin, absolute alcohol, ether, and dried over sulphuric acid, giving a fine white powder soluble in 0.01 *M* KOH.

The following table shows the reactions obtained from these wheat protein preparations. The tests, the results of which are here recorded, were all done under my observation by Dr. I. C. Walker (nos. 1–15 incl.), Dr. Turnbull (nos. 16–19 incl.), Dr. J. L. Goodale (nos. 20–22 incl.), Dr. Fritz B. Talbot (no. 23), and to them am I indebted for the use of their results.

Briefly described, the skin test, by means of which these results were obtained, is done by making small scarifications in the skin of the inner side of the forearm and applying separately to these the different proteins which are then moistened with 0.01 *M* KOH. A reaction is considered positive when an edematous swelling, which is usually surrounded by a red areola, makes its appearance about the scratch within a few minutes after the protein is applied. The intensity of the reaction is gauged by comparison with a control scratch upon which nothing but a drop of 0.01 *M* KOH has been put. For a more complete description of the test the reader is referred to the publications of the above mentioned investigators (Walker, this series no. V '17, Turnbull '16, Goodale '16, Talbot '16).

In recording these results ± is used to indicate a reaction scarcely stronger than the control and should probably very often be regarded as negative; + represents a quite definite reaction and the intensity of the reaction is represented in an arbitrary fashion by the number of plus signs, 4+ representing an edema about the size of a silver dollar. The size of the reactions alone, however, is a very inadequate comparison index of the anaphylactic activity of the proteins in question. For this reason the proteins were dissolved in 0.01 *M* KOH at a concentration of 1 percent and from this solution dilutions were made, in the same medium, in the series 1 : 100, 1 : 1,000, 1 : 10,000, etc., and wherever possible the tests were repeated using the dilutions instead of the dry proteins. Wherever this was done the lowest concentration to give a reaction is recorded in the table together with the size of the reaction.

In all, about seventy patients were tested, but only those are

No.	Patient	Whole Wheat Prot.	Bread Wheat Prot.	Globulin	Gliadin	Glutenin	Leucosin	Nat. Proteose	Art. Pro-teose	Corn Protein	Oat Protein	Rice Pro-tein	Barley Protein	Rye Protein	Wheat Pollen	Corn Pollen
1	G.B. . . .	±		++ 1:100 =0	+	++ 1:100 =0	++ 1:100 =0	+	o	o	o	o	o	o		
2	J.M. . . .	±		+	o	±	o	+		o	o	o	o	o		
3	C.K. . . .	±	+	o	o	o	o	+								
4	C.N.E. .	±	±	++	+	+	+	+	±	o	o	o	o	o		
5	M.S. . . .	+	±	[3]	[3]	[3]	[3]			o	±	o	±			
6	F.A.	+	+	[3]	[3]	[3]	[3]			o	±	±	o			
7	M.S. . . .		o	[3]	[3]	[3]	[3]			+	+	±	o			
8	B.S.	o		o	o	o	+	o	o							
9	P.C.	o		+	±	+	o	o		o	o	o	o	o		
10	P.D. . . .	o		o	o	o	o	+	+	o	o	o				
11	H.M. . . .	+ 1:500	±	++	+	+	± 1:1000	± 1:1000	++	+	o	+	++	+++		
12	F.G.	+	o	±	±	o	+ 1:100	±	±	o	o	o	o			
13	G.B. . . .	±	±	+ 1:100	±	±	±	++ 1:100	±	+ 1:100	+	+	+			
14	D.	++ 1:500	+	+ 1:1,000	+ 1:100	+ 1:100	± 1:1,000	± 1:1,000								
15	H.	++		+	++	+	++	o	o	+		o	±	+		
16	R.	+		o	o	o	+	o	+							
17	F.	o		+	o	o	++ 1:100	++ 1:100	o							
18	B.	+		o	o	o	++ 1:100	+	o							
19	S.	+++	++							+++	+	+	+++			
20	G.	4+	+				++	+	++	o	+	+	+		o	
21	Du.	++					++	+	+	++	++	+	+			+++
22	De.	+								+	+++	++				++
23	N.	+		±	o	o	o	+	±	+						

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[3] Not tested until after treatment when they were found to be negative.

included which gave a positive reaction with one or more of the wheat proteins.

For the sake of comparison with the other cereals, corn, oat, rice, barley and rye proteins were tested, using for this purpose the preparations made by the author (Wodehouse, '16).[2]

The writer is well aware of the incompleteness of the records shown in the following table but this is due to the fact that the investigation was carried out upon patients the number of which reacting to wheat proteins was limited. Then with patients it is not possible to repeat tests with the frequency and thoroughness that is possible when using animals for anaphylactic tests.

When this work was begun the writer was expectant of finding that some one of the proteins of wheat was entirely responsible for its anaphylactogenic properties or else that they all behaved in the same fashion and it was with the idea of isolating the "active principle" that the work was undertaken. That no such simple state of affairs exists can be seen from a glance at the table. On the contrary this work shows that, though all of the proteins are capable of calling forth anaphylactic symptoms, their method of action is so complex that with our present state of knowledge it baffles explanation. Several interesting obtentions to which attention is drawn in the following paragraphs should, however, be noticed.

In many cases where the whole wheat preparation gives a doubtful reaction or even in some where it gives a negative, some one or more of the individual proteins give a quite definite reaction and *always one or more of the individual proteins are as active or, as is usually the case, more so than the whole wheat preparations.*

Of the total number of patients with which any part of wheat gave a reaction

		Percent
Whole wheat protein	reacted with	60
Bread protein	"	45
Globulin	"	57
Gliadin	"	31
Glutenin	"	38
Leucosin	"	61
Natural proteose	"	72
Artificial proteose	"	36

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[2] These preparations were made by soaking the uncooked flour or meal of the cereals in water until a solution rich in protein was obtained. From this the protein material was precipitated by alcohol and the precipitate dried in alcohol and ether.

In computing these figures doubtful reactions were always counted as negative and this accounts for the small proportion (viz., 60 percent) of reactions obtained with whole wheat. Since it is composed of several different proteins this preparation would be expected to have proportionately more chances of producing reactions. On the other hand, however, being a mixture the active proteins would be diluted, to a large extent, by the inactive proteins and with cases with which the active proteins are few in number or weak in reaction this dilution might be sufficient to obscure their activity almost to, or even below, the limit of sensibility of the skin test, thus accounting for the large proportion of doubtful or negative reactions of the whole wheat while with the same cases at the same time the individual proteins reacted quite strongly.

In case No. 10 is seen a good example of this. Here "natural proteose" is the only one active but in the whole wheat preparation it does not call forth a response because its activity is obscured by the other four. With the case of No. 13 "natural proteose" and gliadin are the active parts but their activity is masked by the three other inactive parts. Except to Nos. 1 and 4 this explanation can be applied to all. However it is only tentative and a definite explanation must await further investigation.

An even more interesting result to be observed here is that the "natural proteose" is the most active, producing reactions with 72 percent of the cases while the "artificial proteose" only shows activity towards 36 percent. This shows that these two proteins are not immunologically alike and lends support to the contention of Wells and Osborne (Wells and Osborne, '15) that the "natural proteose" exists preformed in the seed and is not formed, as is the "artificial proteose" by reagents used in extraction and purification.

It is also to be seen that heating to a cooking temperature does not destroy the anaphylactogenic properties of wheat. However the heating employed in the cooking of bread somewhat reduces its activity in most cases. Nevertheless with some the reverse is true. In order to test this further a concentrated watery extract of flour was boiled for several hours. Another was heated in the autoclave at a temperature of 114° C. and a pressure of 15 pounds per square inch for one hour. When the coagula formed by the heat were filtered off and skin tests performed with the filtrates it was found that neither heating to a temperature of 114° C. nor prolonged boiling had reduced their activity in the slightest degree. Nevertheless when the whole seeds of wheat and some of the other cereals were heated in a crucible until they became a light tan color all through, they were found to have completely lost their activity. This was also found to be true of the prepared cereal foods, which are said to be cooked at temperatures much exceeding any reached by an ordinary autoclave, such as "Puffed Wheat," "Puffed Rice," Kellogg's "Toasted Wheat Biscuit," "Shredded Wheat," etc. When concentrated aqueous extracts made from these were tested[4] by means of the skin test upon patients who were strongly sensitive to the corresponding cereals in the raw form no reaction whatsoever was obtained. In this connection it is interesting to note that in the preparation of the most active of the individual wheat proteins (viz., "natural proteose") considerable boiling is employed. From this it is seen that only very high temperatures tend to diminish the anaphylactogenic activity of wheat and of some of the other cereals in relation to sensitization as revealed by the skin test.

In cases sensitized to the pollens of the Gramineae it has been pretty definitely shown that idiosyncrasy to pollen of one species of grass is almost always accompanied by sensitization to the pollens of all the grass family (Goodale, '15). However, in cases allergic to the seed proteins of the Gramineae we see that this is not generally so, though sometimes it may be, especially with cases highly sensitized. This is entirely in keeping with Nuttall's findings in the immunological relationships among the animal proteins. He says: "The more powerful the antiserum obtained the greater its sphere of action upon the bloods of related species. For instance, a weak anti-human serum produced no reaction with the blood of the Hapalidae, whereas a powerful serum did produce a reaction" (Nuttall, '01). This is confirmed by Uhlenhuth (Uhlenhuth, '01) in experiments upon the relationships between the ox, goat and sheep.

The question as to what extent subjects which are hyper-sensitive to the seed proteins of the Gramineae respond to the pollens of this family should be further investigated. It is interesting to note in passing that wheat pollen was entirely negative with the one case upon which it was tried although this case was extremely sensitive to the proteins of the seed. The same preparation of wheat pollen, however, gave good reactions with some grass hay-fever cases.

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[4] These tests were made by Dr. J. L. Goodale with the materials prepared by the author.

SUMMARY

The five proteins globulin, gliadin, glutenin, leucosin and natural proteose were prepared from wheat according to the method of T. B. Osborne, and when they were compared in their anaphylactogenic properties with each other, with an artificial proteose prepared by hydrolysis from glutenin, with the whole wheat preparations and with the proteins of other cereals, it was found that (1) all are anaphylactogenic, but no two are immunologically exactly alike, (2) the natural proteose is the most active, (3) the natural proteose is different from the artificial proteose, (4) in any given case where whole wheat gives a reaction and in some where it does not some one or more of the individual proteins are sure to be found to be more active, (5) it does not necessarily follow that because a case is allergic to wheat it will be found to be also hypersensitive to the other cereals (though this is sometimes the case especially if sensitization is of a high order), (6) it probably does not follow that sensitization to the seed proteins of cereals necessitates sensitization to the pollens of the same species, though not enough experiments were done upon this to more than suggest that it is a problem that ought to be further investigated.

It is also shown that heating, except to very high temperatures, does not materially affect the anaphylactogenic properties of the wheat proteins.

MEDICAL CLINIC OF THE
PETER BRIGHAM HOSPITAL,
BOSTON, MASS.

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THE TOXICITY OF GALACTOSE AND MANNOSE FOR GREEN PLANTS AND THE ANTAGONISTIC ACTION OF OTHER SUGARS TOWARD THESE[1]

Lewis Knudson.

In experiments concerned primarily with the utilization of certain sugars by certain green plants (Knudson, 1915, 1916), the noteworthy fact developed that, while other sugars may be of benefit, galactose is toxic. The injurious effect was manifested in a killing of the root or a retardation of root growth, depending upon the concentration of galactose employed. It was observed, furthermore, that glucose can antidote the toxicity of galactose, but this antagonism occurs only when the glucose is present at a concentration equal to or greater than that of the galactose. So far as the writer has been able to determine, this is the only recorded case of a hexose sugar being injurious to plants and of antagonism among the sugars.

In view of the fact that glucose exhibited such a marked antagonistic action toward galactose, it seemed advisable to extend further the investigation to include various other sugars. A considerable number of experiments have been made to this end and the paper here presented records briefly the results obtained.

Methods.—For the experiments either Canada field pea (*Pisum arvense* L.) or wheat (*Triticum sativum* L.) was used. The plants were grown in all cases under conditions insuring freedom from microorganisms. For this purpose the plants were grown in culture tubes 200 mm. × 20 mm. in size, on a nutrient agar medium. Pfeffer's nutrient solution, slightly modified, was made up as follows: $Ca(NO_3)_2$, 4 grams; KNO_3, 1 gram; K_2HPO_4, 1 gram; KCl, 0.5 gram; $MgSO_4$, 0.5 gram; $FeCl_3$, 20 milligrams; distilled water, 12 liters. The agar used had been previously rinsed three times in distilled water and then air-dried. One percent of agar was used. The medium is faintly alkaline to methyl red. The different sugars were dissolved in this medium and stock solutions were made up of double the concentration of sugar used in the experiments. Dilution of the sugar was effected by addition to the nutrient solution or by addition of another sugar solution. For example, to obtain a solution containing 0.25 mol. galactose + 0.025 mol. saccharose, it was necessary to mix equal parts of 0.5 mol. galactose and 0.5 saccharose. The volume of the medium in each tube was 25 cc. Sterilization was effected by autoclaving at fifteen pounds pressure for fifteen minutes.

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[1] Contribution from the Laboratory of Plant Physiology, Cornell University.

All the sugars used, with the exception of arabinose, were supplied by Dr. C. S. Hudson, in charge of the carbohydrate laboratory, U. S. Bureau of Chemistry, and are stated by him to be of very high purity. The arabinose used was a Merck reagent.

Character of the Injury.—The injurious action of galactose is made evident first in the roots. The primary root coming in contact with the agar may first become brown and in a few days death results. In other cases the tip of the root is killed and this stimulates the production of a large number of lateral roots, the tips of which, on coming in contact with the agar medium, are soon killed. A short primary root with many laterals results, the appearance of which is somewhat centipedal. Two plants in the same culture may, however, vary in the manner of injury, and the presence of certain sugars may alter the extent of the injury.

For the sake of clearness and definiteness, it seems desirable to describe the injury by a numerical system as well as by root lengths. Accordingly the following key is given: 0, no injury; 1, primary root tip killed, laterals not injured; 2, the primary root tip may be killed, but the laterals may attain a length of a few centimeters and then growth is stopped or the roots are killed; 3, the primary root may penetrate the agar, but becomes brownish and five or six centimeters long; 4, the primary root may attain a length of a few centimeters, but becomes brown in color and the laterals do not grow beyond 0.5 cm.; 5, the primary root tip is killed and all laterals suffer likewise; 6, the primary root is entirely killed.

Antagonistic Action.—In the following experiment the galactose was supplied at a concentration of 0.025 mol. and the other sugars were used at the same concentration. In order to demonstrate conclusively that the total concentration was not responsible for any toxicity, a few cultures were made with the nontoxic sugars supplied at 0.05 mol. The experiment was begun on January 29, 1917, and concluded on February 13, 1917. The cultures were placed in the greenhouse and grown in the light. All cultures were made in triplicate, but contamination or failure to germinate caused a loss of some of the cultures. The seed were sterilized by immersion in a solution of calcium hypochlorite (calcium oxychloride, Baker) according to the method of Wilson (1915). The peas were treated for two hours and the wheat for five hours. The results are given in Table 1.

TABLE 1

Influence of Sugars on the Toxicity of Galactose

The Concentration of Each Sugar Equals 0.025 Mol.	Length of Primary Root (Cm.)	Average Length of Lateral Root (Cm.)	Length of Top (Cm.)	Class of Injury
Galactose (3 cultures)	1	½	6	5
Galactose (2 cultures)	0	0	3	6
Glucose (3 cultures)	10	8	15	0
Levulose (3 cultures)	8	8	14	0
Arabinose (1 culture)	10	7	15	0
Saccharose (3 cultures)	9	8	14	0
Maltose (3 cultures)	10	8	13	0
Raffinose (2 cultures)	9	8	14	0
Pfeffers, no sugar (3 cultures)	10	8	14	0
Galactose+glucose (3 cultures)	9	7	13	0
Galactose+levulose (2 cultures)	5½	¼	10½	4
Galactose+levulose (1 plant)	7	0	....	5
Galactose+saccharose (3 cultures)	3	7	13	1
Galactose+saccharose (1 culture)	10	9	14	0
Galactose+lactose (3 cultures)	1	½	9	5
Galactose+maltose (2 cultures)	1	½	10	5
Galactose+raffinose	1	½	6	5

From the table it will be noted that the toxicity of galactose is prevented by glucose or saccharose, the former being slightly more effective than the latter since the primary root is not killed in the presence of glucose. None of the other sugars are effective in preventing the injurious action of galactose, although in the presence of levulose the primary root may continue its growth to a limited extent. Representative cultures are shown in Fig. 1.

All of the preceding experiments except those with levulose were repeated and similar results were obtained.

In some earlier experiments (Knudson, 1916) it was noted that glucose does not antidote galactose if the concentration of the former is less than that of the latter. It was thought that some relation might be found between concentrations and antagonistic action. Accordingly the galactose was supplied in each case at a concentration of 0.0125 mol. solution, and the other sugars used at double this concen-

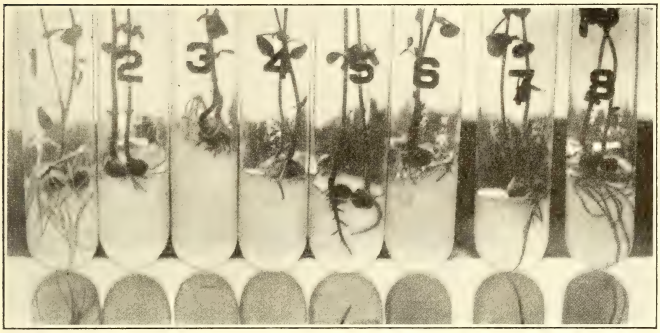

FIG. 1. 1. Galactose .025 mol. + saccharose .025 mol.
2. “ “ “ + maltose .025 “
3. “ “ “ + raffinose .025 “
4. “ “ “ + lactose .025 “
5. “ “ “ + arabinose .025 “
6. “ “ “
7. “ “ “ + levulose .025 “
8. Pfeffer’s solution. No sugar.

TABLE 2

Influence of Sugars on the Toxicity of Galactose

Culture Solution	Length of Primary Root (Cm.)	Average Length of Lateral Root (Cm.)	Length of Top (Cm.)	Class of Injury
Galactose .0125 mol. (2 cultures)	1	0.5	7	5
Galactose .0125 mol.+glucose .025 mol. (1 culture)	9	8	15	0
Galactose .0125 mol.+lactose .025 mol. (1 culture)	7	1.5	12	3
Galactose .0125 mol.+levulose .025 mol. (1 culture)	3	6	13	3
Galactose .0125 mol.+arabinose .025 mol. (1 culture)	5	1	13	4
Galactose .0125 mol.+saccharose .025 mol. (1 culture)	2	8	15	1
Galactose .0125 mol.+lactose .025 mol. (2 cultures)	6	0.5	10	4
Galactose .0125 mol.+lactose .025 mol. (2 cultures)	1	1	12	5
Galactose .012 mol.+maltose .025 mol. (2 cultures)	1	0.5	12	5
Galactose .0125 mol.+raffinose .025 mol. (2 cultures)	1	0.5	10	5

tration, or 0.025 mol. It was noted that 0.0125 mol. galactose is as toxic as 0.025 mol. galactose. The results are given in Table 2. Since there was variation in some of the series, the results of individual cultures are recorded in these cases; for plants that were alike, the averages are recorded.

In general the results are similar to the preceding experiment, though levulose is somewhat more effective than with the higher concentrations of galactose. Cultures are represented in Fig. 2.

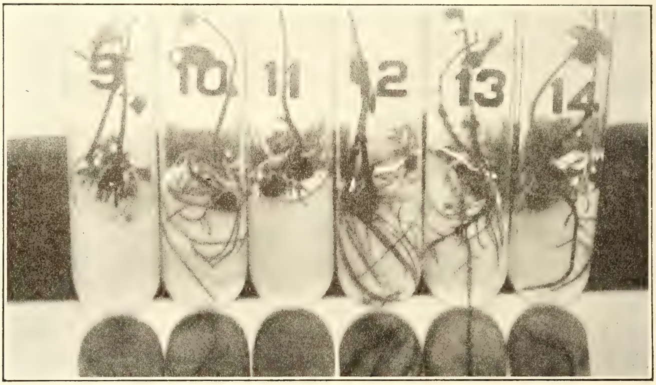

FIG. 2. 9. Galactose .0125
10. " + saccharose .025
11. " + raffinose .025
12. " + glucose .025
13. " + levulose .025
14. " + lactose .025
15. Lactose .05
16. Pfeffer's solution. No sugar.

Toxicity of Mannose.—In the course of certain experiments on the use of various sugars by vetch (*Vicia villosa*) grown in water cultures, it was noted that mannose, supplied at a concentration of 0.025 mol., killed the tips of roots that came into contact with the solution. Experiments were then made with pea and wheat to determine whether the effect was consistent, and agar cultures were used as in the experiments previously described with galactose. It was found that mannose at a concentration of 0.025 mol. behaved very much as did galactose, with the possible exception that the browning of the roots was not so intense as with galactose. The same general effect, however, was noted as is evident in Figs. 3 and 4.

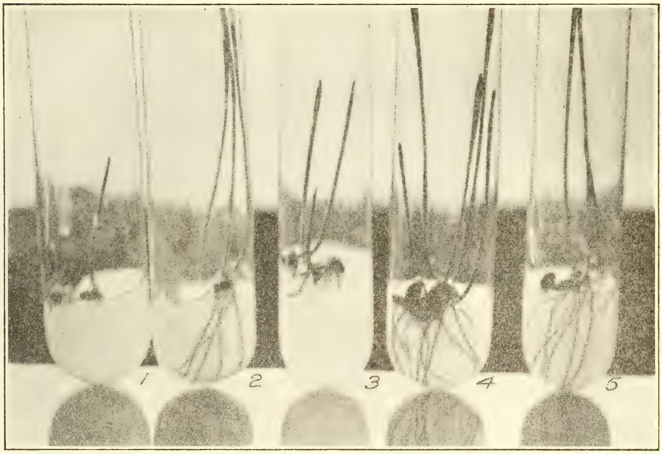

FIG. 3.
1. Galactose .025 mol.
2. Mannose .025 "
3. Mannose .025 " + galactose .025 mol.
4. Mannose .025 " + glucose .025 "
5. Pfeffer's solution. No sugar.

Antagonistic Action.—Since glucose and saccharose had been found to antidote effectively galactose, these two sugars were tested with respect to their preventing the toxicity of mannose. Test-tube cultures were used as for the galactose experiments, the amount of the medium being 25 cc. Both peas and wheat were used. The cultures were permitted to grow for two weeks, and the data were then recorded. All cultures were in duplicate, and those for pea, with the exception of the cultures containing saccharose, were repeated.

The cultures employed were as follows:

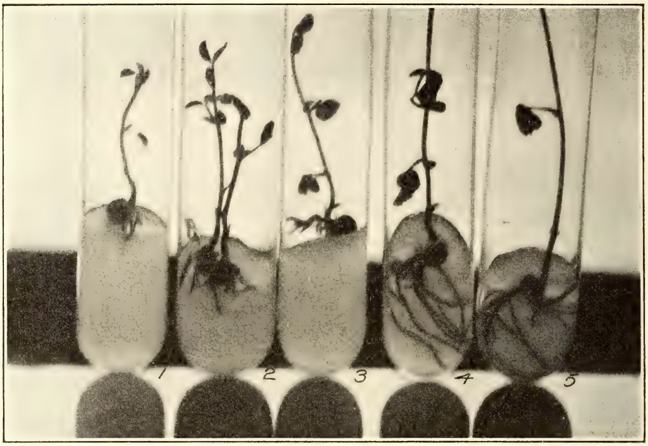

Fig. 4. 1. Galactose .025 mol.
2. " .025 " + mannose .025 mol.
3. Mannose .025 mol.
4. " .025 " + glucose .025 mol.
5. " .025 " + saccharose .025 mol.

1. Galactose .025 mol.
2. Mannose .025 mol.
3. Mannose .025 mol. + galactose .025 mol.
4. Mannose .025 mol. + glucose .025 mol.
5. Mannose .025 mol. + saccharose .025 mol.
6. Glucose .025 mol.
7. Saccharose .025 mol.
8. Pfeffer's solution alone—no sugar.

Both with wheat and with peas it was found that the toxicity of mannose is antidoted by either glucose or saccharose. Mutual antagonism was not found between galactose and mannose. The plants grown in the other solutions were in every way normal. Representative cultures are shown in Figs. 3 and 4.

Discussion.—The hexose sugars glucose, mannose, and galactose are stereoisomers. All of them are used by various fungi, and mannose is as readily fermented by yeasts as is glucose. Galactose, however, is fermented with greater difficulty, and it is suggested (Armstrong, 1912) that perhaps a different mechanism is involved in its fermentation. Mannose has a common enolic form with glucose and fructose, and any one of the three may be converted into any other under the influence of alkalies. It is therefore all the more surprising to find mannose behaving similarly to galactose and not like glucose.

In a previous paper it was suggested that the toxicity of galactose might be due to its oxidation products. The first oxidation products of glucose and galactose are gluconic and galactonic acids. Various cultures were made with Canada field pea in which the effect of calcium galactate and calcium gluconate was to be noted. The experiments were made as were those previously described. In no case was any injurious action of calcium galactate noted.

It is not yet possible to offer any explanation accounting for the toxicity of the two sugars. An explanation of antagonism is suggested by the phenomenon commonly observed with fungi, namely, the election of organic substances, whereby if two organic substances are offered only one may be absorbed. Various cases of this nature have been reported even for stereoisomeric compounds. According to this view, in a mixture of glucose and galactose, the toxicity of the latter would be prevented because of the absorption of glucose and the nonabsorption of galactose, and a similar condition would hold for a mixture of saccharose and galactose. The failure of the other sugars to antagonize the toxicity of galactose would be due to their inability to prevent the absorption of the galactose. Work is being continued on this subject.

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CONTENTS

Taxonomic characters of the genera Alternaria and Macrosporium. John A. Elliott 439

Crown-rot of fruit trees: histological studies........J. G. Grossenbacher 477

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TAXONOMIC CHARACTERS OF THE GENERA ALTERNARIA AND MACROSPORIUM

JOHN A. ELLIOTT

I. INTRODUCTION

Alternaria and Macrosporium are among the most universally distributed and most common forms of the Fungi Imperfecti, embracing, according to the "Sylloge Fungorum" of Saccardo, 41 species and varieties of Alternaria and 189 species and varieties of Macrosporium, these numbers including some synonyms but not the new species which have been described since the publication of the last volume of Saccardo's work. Some species, such as *Alternaria solani* (E. & M.) J. & G., *A brassicae* var. *nigrescens* Peglion, and *Macrosporium sarcinaeforme* Cav. are well known and destructive parasites, but the great majority are saprophytes or have been described from non-important hosts. The ascigerous stages of a few species are known, the connection in all such cases being with the genus Pleospora.

Even a casual survey of the literature dealing with the genera in question would reveal the fact that the generic names, Alternaria and Macrosporium, are in many cases used synonymously in dealing with the best known of the parasitic species. This condition could be due either to there being no basis for distinction between the two genera, or to this basis being ill defined. The studies of the writer were undertaken with the hope of adding to the knowledge of these two genera. The work was necessarily limited and the result is in no way of the nature of a monograph.

II. HISTORICAL

The genus Alternaria was described and figured by Nees (15), *A. tenuis* being the type and only species described. The description

[The **Journal** for July (4: 375–438) was issued July 14, 1917]

is incomplete and in some particulars inaccurate, but it is definite and complete enough to leave little doubt that what Nees described was what is now generally recognized as Alternaria.

Fries (7) described the genus Macrosporium, differentiating it from Cladosporium, Helminthosporium, and Sporodesmium. The muriform spore, now given as one of the characters of the genus, is not mentioned in the generic description, otherwise it fits the present current conception fairly well. Having dropped the genus Alternaria, Fries makes no mention of it in his description of Macrosporium.

Macrosporium and Alternaria are placed by reason of their muriform spores in the section Dictyosporae of the family Dimidiaceae of the order Moniliales, the muriform spores separating them from the genera Cladosporium and Helminthosporium, which in some species are in many particulars similar. Among the Dictyosporae there is little basis, as the genera are described, for separating Stemphylium, Septosporium, or Mystrosporium from Macrosporium. The separation of the genera Alternaria and Macrosporium rests solely on the catenulation of spores in the former genus. The fact that many of the species of Alternaria now recognized were first described as Macrosporiums indicates the uncertainty of this basis for generic distinction. In the specific descriptions in both genera, while mycelium, conidiophores, and spores may all be taken into consideration, spore characters are the most used basis for distinction.

The question of the validity of the separation of the two genera arose over the study of their ascigerous connection with *Pleospora herbarum* Tul. The Tulasne brothers (19) figure *P. herbarum* bearing both Alternaria and sarcinaeform spores on the same hyphae. Gibelli and Griffini (8), Mattirolo (13), Bauk (1), and Kohl (12), studying *P. herbarum* in pure culture, concluded that it should be divided into two varieties or species, one having Alternaria conidia and the other having sarcinaeform conidia. Miyake (14), studying the life history of *Macrosporium parasiticum* Thum., found no Alternaria stage in the life cycle. Halsted (9), in studying the life history of *Pleospora tropaeoli* Hal. in pure culture, found that the cycle included only Pleospora and Alternaria stages.

As the ascigerous stage of most species of Alternaria and Macrosporium is unknown or non-existent, the basis for the distinction of genera and species must rest, in general, on the conidia. Jones (10–11), in studying *Macrosporium solani* E. & M. and *M. fasciculatum* C. & E.

on artificial media, found that they formed chains of conidia and placed them in the genus Alternaria. Bioletti (3) reported securing pure cultures of *Macrosporium* sp. and *Alternaria* sp. from olives in California. Others have reported isolating species of one or the other genera from various sources and growing them in pure culture under conditions where the morphology of the fungi ought to have been pretty certainly ascertained, but by far the greatest amount of literature on the two genera deals with their pathogenic effects or with the mere description of species.

Miyake (14), Prillieux and Delacroix, (17), and others (4) have shown by careful experiments or have suggested that many of the specific names are synonyms. Constantin (4) and Planchon (16) have reported great variations in Alternaria due to growth on different media. Planchon (16) expresses the opinion that Macrosporium is merely Alternaria with dissociated conidia. No extensive comparative work, either between the two genera or between species of the two genera, has yet been undertaken.

III. METHODS IN GENERAL

To aid in the comparison of published descriptions, a tabulation of the species and varieties of Alternaria and Macrosporium given in the "Sylloge Fungorum" was made on the basis of spore length, the species being arranged according to the maximum length given. Specimens from all available exsiccati were studied and compared. Cultures of the principal types found in the exsiccati were studied under varied conditions in order to learn something of the constancy of the characters which are made the basis of specific distinctions. The original generic descriptions were studied in order to ascertain the basis of generic distinction.

The Value of Written Descriptions

An examination of the specific descriptions of the two genera in question showed that spore measurements were most constantly used in distinguishing between species; in many cases several species were alike in every character given except that of size of spores. In order to learn something of the variation in measurements which may be due to the personal element and to the use of different micrometers and microscopes in measuring, three slides were prepared and together with cultures of *Phoma destructiva* Pat. were sent to a number of persons actively engaged in descriptive mycological work. On one slide of *Pleurosigma angulatum* Sm., a single frustule was enclosed in a circle and indicated; on a second slide two spores of *Alternaria fasciculata* were similarly indicated. A third slide of *A. fasciculata* contained several hundred spores. Identical typewritten directions were sent to each one who made the measurements, asking that no more care be used than would ordinarily be taken in measuring for the purpose of describing a new species. A tabulation of results follows:

TABLE A.

Observer No.	Eyepiece Value	Single Frustule of *P. angulatum*	One Spore of Alternaria	One Spore of Alternaria	Many Spores of Alternaria	Many Spores of Phoma
1	2.4	285.6×45.6	48×7.2	19.2×9.6	12–36×6–12	2–5×1–2
2	2.4	284.4×46.8	49.2×6	19.2×9.6	14–36×9–12	3–4×2–3
3	1.8	284.4×45.9	50.6×7.2	19.8×9.9	11–41×6–13	
4	3.16	282×46.5	48×7.5	19.5×9.5	10.5–24×7.5–13	5–6×1.5–2
5	3.4	282×46	48×7	20×10	17–34×7–10	
6	3.2	280×45	48×7	20×7	13–29×9–11	4–8×2–3.5
7	4.	280×44	48×7	20×9	20×9	5–6×2–2.5
8	3.2	276×45	48×8	21×7	12–35×9–12	5–9×2–3
9	10.	275×50	......	......	13–35×7–11	......
10 ...	3.2	275×45	47×7	20×10	12–33×6–12	3–8×2–3
11 ...	3.	270×48	50×7.5	21×9.5	15–40×8–12	5–8×3–5
Variation ...		15.6×6	3.6×2	1.8×3	6.5–17×3–3	3–4×2–3
Variation %.		5×12	7×25	8.5×30	43–41×33–23	60–44×66–60

The second column gives the value of the smallest division of each eyepiece micrometer in micromillimeters. All the measurements given in the table are in microns. The arrangement is according to the maximum measurement given for *Pleurosigma angulatum.*

The third, fourth and fifth columns show the variations in measurements when all, without any doubt, were measuring the same things. The variation is least in the longest measurements, being a little over 5 percent in the greatest length given, and greatest in one of the two shortest measurements, amounting to 30 percent in the width of the spore indicated in the fifth column. That this variation is not due to the eyepiece used is shown by the fact that observer No. 10 who returned the lowest measurement for the long spore, column *4*, also returned the highest measurement for the width of the spore given in column *5*. The variations given for the Phoma spores are the greatest, being 66 percent for the shortest measurement and 44 percent for the longest measurement. In this case each mycologist made his own microscopic preparation.

The most instructive results appear in the sixth column of the table where the maximum and minimum lengths and breadths of the spores are given under conditions such as would obtain in describing a new species. Here there was a variation of over 41 percent of the highest maximum measurement returned for the length of the spores. The variation for the minimum lengths was greater. Assuming variation equal to that shown in column *6* of Table A, 34 species of Macrosporium and 5 species of Alternaria in the "Sylloge Fungorum" are inseparable by measurements of both length and breadth.

Applying to all the species of Alternaria and Macrosporium in the "Sylloge Fungorum" the variability shown in column *6* of Table A, they can be combined into thirteen groups, taking into consideration the measurements for both length and breadth of spores. In other words, if other characters are disregarded, in so far as actual spore measurements are dependable, there are only thirteen species of Macrosporium and Alternaria adequately described in the "Sylloge Fungorum."

Study of Exsiccati

Following the study of descriptions in the "Sylloge Fungorum," examination was made of the specimens of Macrosporium and Alternaria in the exsiccati immediately available. One hundred and thirty-four specimens labeled as 85 species, were found. Of this number 17 were marked "sp. n." *i. e.*, of or nearly of the value of type material. Eighteen other species not marked "sp. n." were found in the exsiccati of the authors or one of the joint authors of the species. This gave a total of 35 species, the material of which can be regarded as reasonably authentic.

Mounts were made from each of the specimens, from which spore and conidiophore measurements, and the character of each, were recorded. The nature of the growth, whether apparently parasitic or saprophytic, was also recorded and the descriptions so made were compared. There was no doubt that in the collection, many specimens morphologically indistinguishable appeared under different names, and that in some instances the same name was given to specimens which were in no way similar or which could readily be distinguished from each other.

In the following summary of the study of the exsiccati the names are given as they appeared on the specimen, followed by the title of the collection and the specimen number in the collection. Two asterisks (**) following the species name indicates that the specimen was marked "sp. n."; a single asterisk (*) indicates that the specimen was found in the exsiccati of the author of the species.

Group 1

The following species had only globular or packet-shaped spores and were essentially alike:

*Macrosporium sarcinaeforme** Cavara, Fungi Par., Bri. & Cav., 116.
M. cladosporioides Desm., Fungi Sel. Ex., Roum., 5596.
M. stilbosporoideum Bri. & Cav., N. Amer. Fungi, Ellis, 2080.

Group 2

A second group was made of those having globular or packet-shaped spores like those of the first group, but having in addition some ovate or pointed spores which might be due to variation in the shape of spore or to a mixture of two forms:

*M. parasiticum*** Thum., Myc. Univ. Thum., 667; Fungi Par., Bri. & Cav., 152.
*M. consortiale*** Thum., Myc. Univ. Thum., 1373.
M. sarcinula Berk., Fungi Columb., 3032.
M. chartarum Pk., N. Amer. Fungi, Ellis, 648; Fungi Sel. Ex., Roum., 6560.
M. heteronemum (Desm.) Sacc. Fungi Sel. Ex., Roum., 6647, 6562, 6358.

The following were possibly the same as the above but they either showed minor differences or else the material was not sufficient to afford positive judgment:

M. chartarum Pk., Fungi Columb., 396.
M. zimmermaneii Thum., Fungi Sel. Ex., Roum., 396.
M. polytrichum Cke. & Rav., Fungi Par., Bri. & Cav., 191.
M. puccinioides E. &. And., N. Amer. Fungi, Ellis, 2876.

Group 3

A third group was made of species having long, narrow, regular, tapering spores with few longitudinal septa. All were apparently parasitic:

*M. euphorbiae*** Bart., Fungi Columb., 2633.
*M. carotae** E. &. E., N. Amer. Fungi, Ellis, 3289; Fungi Columb., 2632.

M. amaranthi Pk., Fungi Columb., 2631.

M. brassicae f. solani Faut. et Brun., Fungi Sel. Ex., Roum., 6559.

M. cheiranthi Fr., Fungi Amer., Rav., 303.

M. solani E. & M., Fungi Columb., 891; 398; 3635; Fungi. Par., Bri. & Cav., 191; Economic Fungi, Sey. & Earle, 339; 340; N. Amer. Fungi, Ellis, 1265; 2485.

Alternaria solani (E. & M.) J. & G., Econ. Fungi, Sey. & Earle, 521.

A. brassicae (Berk.) Sacc., Fungi Par., Bri. & Cav., 87.

Two forms similar to the above were:

*M. caudatum** C. & E., Fungi Columb., 397. This was like the above but uniformly shorter.

M. antennaeforme B. & C., Fungi. Columb., 2531. This species was in general form similar to those in the above group but the spores were uniformly more slender.

Group 4

Three other specimens were found with spores of the same form as the above group but the spores were much larger. These were:

*M. herculeum** E. &. M., N. Amer. Fungi, Ellis, 1263.

*M. commune** Rabh., Fungi Europ., Rabh., 1360.

M. saponariae Pk., N. Y. Fungi, Shear, 397; Fungi Sel. Ex., Roum., 3868.

Group 5

Another group was formed of species with spores similar to those of *Alternaria solani* but generally wider and shorter and always more markedly muriform. The species in this group are not morphologically identical but merely similar.

*M. heteroschemon*** Faut., Fungi Sel. Ex., Roum., 6942.

*M. cucumerinum** E. &. E., N. Amer. Fungi, Ellis, 3396.

M. nervii Cke., N. Amer. Fungi, Ellis, 964.

M. sucaviae Trabut., Fungi Sel. Ex., Roum., 4098.

M. convallariae Fr., Fungi Sel. Ex., Roum., 1897.

M. brassicae Berk., Fungi Sel. Ex., Roum., 6442.

M. martindalei E. &. M., N. Amer. Fungi, Ellis, 1262; Fungi Europ., Rabh., 3282.

M. cheiranthi Fr., Fungi Sel. Ex., Roum., 7235.

*Alternaria malvae** Roum., Fungi. Sel. Ex., Roum., 3393.

A. brassicae (Berk.) Sacc., Econ. Fungi, Sey. & Earle, 515.

Group 6

In the following group the specimens were essentially all alike; quite variable in color, shape, and size. The color varied from light to dark olive both in spores and conidiophores. Almost all appeared to be growing saprophytically.

*M. hibiscinum*** Thum., Myc. Uni., Thum., 979.
*M. baptisiae*** Thum., Myc. Uni., Thum., 1271; Fungi Sel. Ex., Roum., 4897.
*M. cassiaecolim*** Thum., Myc. Uni., Thum., 1270; Fungi Sel. Ex., Roum., 4795.
*M. gossypinum*** Thum., Myc. Uni., Thum., 1469; Fungi Sel. Ex., Roum., 4898.
*M. ravenelii*** Thum., Myc. Uni., Thum., 2071; Fungi Sel. Ex., Roum., 4680.
*M. rubi*** Ellis, N. Amer. Fungi, Ellis, 544.
*M. truncatum*** Laub. & Faut., Fungi, Gallici, Roum., 6752.
*M. inquinans** C. & E., N. Amer. Fungi, Ellis, 369.
*M. ornatissimum** E. & B., Fungi Columb., 1741.
*M. porri** C. & E., Fungi Columb., 1279; N. Amer. Fungi, Ellis, 370.
*M. caudatum** C. & E., Fungi, Amer., Rav., 607; Fungi Columb., 890; Fungi Columb., 397; N. Amer. Fungi, Ellis, 816.
*M. iridis** C. & E., N. Amer. Fungi, Ellis, 51.
*M. canificans** Thum., inid, Myc. Uni. Thum., 2280; Fungi. Gall. Ex., Roum., 4794.
*M. leguminum** Cke., Fungi Amer., Rav., 300; Fungi Amer., Rav., 603.
*M. maydis** C. & E., N. Amer. Fungi, Ellis, 3098; Rabh.-Winter, Fungi Europ., 3592.
*M. catalpae** E. & M., N. Amer. Fungi Ellis, 1264; Econ. Fungi, Sey. & Earle, 144.
*M. martindalei** E. &. M., N. Amer. Fungi, Ellis, 1262.
M. tomato Cke.,* N. Amer. Fungi, Ellis, 2484; Fungi Amer., Rav., 603.
M. tenuissimum Fr., Myc. Uni., Thum., 980.
M. convallariae Fr., Myc. Uni., Thum., 1965.
M. chartarum Pk., Fungi Columb., 396.
M. floridanum Cke., Fungi Amer., Rav., 299.
M. florigenum Ell. & Dear., N. Amer. Fungi, Ellis, 3097.
M. togenariae Thum., Fungi Columb., 1367.
M. clematis Pk., Fungi Columb., 1830.
M. bulbotrichum Cke., Fungi Amer., Rav., 604.

M. erumpens Cke., Fungi, Amer., Rav., 605.
M. graminum Cke., Fungi Amer., Rav., 606.
M. caespitulus Cke., Fungi Amer., Rav., 906.
M. cheiranthi Cke., Fungi Sel. Ex., Roum., 4490.
M. consortiale Thum., Fungi Sel. Ex., Roum., 4992.
M. saponiariae Pk., Fungi Sel. Ex., Roum., 3868.
M. phomoides Thum., Fungi Sel. Ex., Roum., 6145.
M. caespitulorum Rabh., Fungi Sel. Ex., Roum., 7236.
M. fasciculatum C. & E., Fungi Sel. Ex., Roum., 1058; N. Amer., Fungi, Ellis, 52; Fungi, Columb., 399; Myc. Uni., Thum., 1870.
M. commune Rabh., Fungi Sel. Ex., Roum., 2068, 4239, 3288, 6443; Fungi Amer., Rav., 304; Fungi Columb., 2330; N. Amer. Fungi, Ellis, 418.
Alternaria fasciculata (C. & E.) J. & G., Econ. Fungi, Sey. & Earle, 522; Fungi Columb., 1368.

Except for being lighter colored, *M. peponicolum*** Rabh., Fungi Europ., Rabh., 1285, was like those in the above group. Several others were in most particulars like the above but either differed in some respects or else the material was too scanty for judgment. These were:

*M. abruptum** C. & E., N. Amer. Fungi, Ellis, 127; Fungi Amer., Rav., 302.
*M. phaseoli*** Faut., Fungi Sel. Ex., Roum., 6247.
*M. cercosporoides** C. &. E., Fungi Columb., 1740.
*M. valerianellae** Roum., Fungi Sel. Ex., Roum., 3690.
*M. elegantissimum** Rabh., Fungi Europ., 2883; Fungi Sel. Ex., Roum., 2067.
M. concinum B. & Br., Fungi Sel. Ex., Roum., 6443.
M. commune Rabh., Fungi. Sel. Ex., Roum., 4240.
M. puccinioides E. & And., Fungi Columb., 1172.

Group 7

Two quite similar species which differed from any other specimens were:

*M. junci*** Lamb. & Faut., Fungi Sel. Ex., Roum., 6444.
M. brassicae Berk., Fungi Sel. Ex., Roum., 2363; N. Amer. Fungi, Ellis, 2483.

They were like *Alternaria brassicae* var. *microspora*, which the latter undoubtedly was.

One species, *A. cucurbitae*** Let. & Roum., Fungi Sel. Ex., Roum., 3694, did not afford enough material for judgment.

The above study of exsiccati and descriptions brings not only species into question but genera as well, since in all but the first and second of the above groups both Alternaria and Macrosporium are included in groups as morphologically similar.

IV. EXPERIMENTAL RESULTS

Cultures of Alternaria and Macrosporium and material upon which either was growing were secured from many sources.[1] Of eighty cultures thus obtained all but two produced chains of spores regularly on artificial media and accordingly belonged in the genus Alternaria. All of these had clavate, elongate or ovate, more or less pointed spores. The two which did not ordinarily produce chains of spores had globular or sarcinaeform conidia. One of these very rarely produced chains of two spores, in which cases the bottom spore was pointed. Eleven of the cultures were selected as representative of all the forms present and as most suitable for extensive study. These eleven cultures also represented all of the morphological forms found in exsiccati. They were: *Alternaria solani* (E. & M.) J. & G., isolated from blighted potato leaves (*Solanum tuberosum* L.); *A. solani* isolated from Datura leaf spot (*Datura stramonium* L.); *A. brassicae* var. *nigrescens* Peglion, isolated from blighted cantaloupe leaves (*Cucumis melo* L.); *A. bras-*

EXPLANATION OF GRAPHS 1 TO 9

The measurements of spores are indicated in microns by the base line, each space representing one μ. The frequency is indicated on the perpendicular lines, each space representing one spore. Measurements are at intervals of 2.4 μ except in the case of the narrower spores where the width is taken at intervals of 1.2 μ.

The following letters are used to indicate the host and media: *A*, natural host; *B*, bean agar, 30°; *C*, bean agar, 10°; *D*, bean agar, + 20 Fuller's scale; *E*, bean agar, − 20 Fuller's scale; *F*, synthetic agar; *G*, synthetic agar minus glucose; *H*, synthetic agar with double amount of glucose; *I*, synthetic agar with double amount of asparagin; *J*, synthetic agar without asparagin; *K*, leached agar.

The most striking facts to be observed are: general reduction in size of spores on the synthetic agars (*F* to *J*), over that on bean agars (*B* to *E*). Relative constancy of species given in graphs 2, 4, 5, and 6 over those given in graphs 3, 7, 8, and 9. Increase in size of spores at lower temperature (Graphs 7 and 9; *C*). Extreme range of variation in size of larger spores (graphs 1, 2, 3, 8 and 9).

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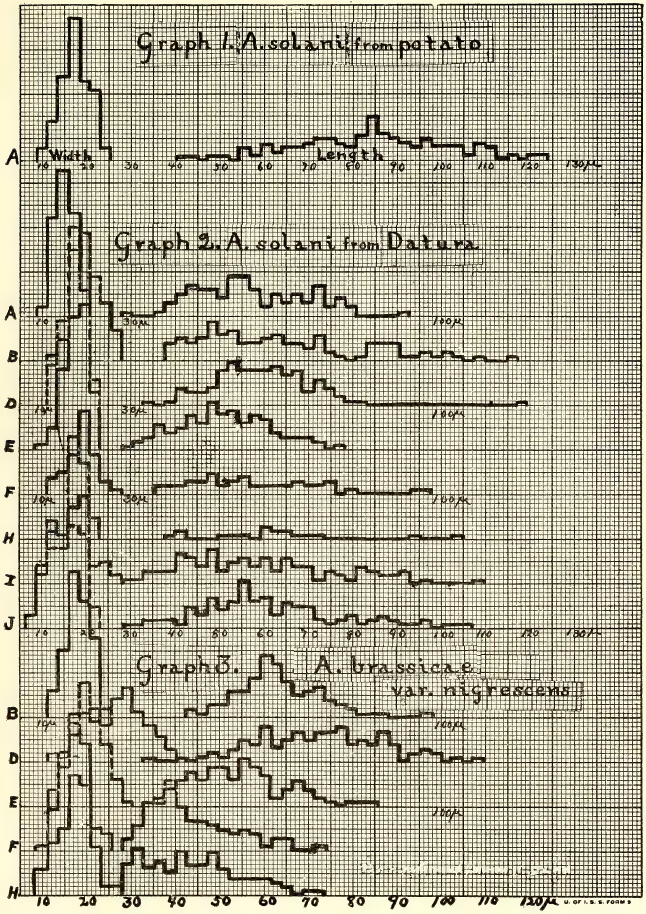
[1] Cultures or material was received from J. J. Davis, L. R. Jones, B. F. Lutman, W. G. Sackett, S. M. Tracy, H. T. Güssow, E. C. Stakman, G. P. Clinton, M. T. Cook, G. F. Atkinson, B. D. Halsted, I. M. Lewis, C. W. Edgerton, C. R. Orton, B. B. Higgins, J. W. Eastham, Miss Jean MacInnes, G. L. Peltier, F. C. Stewart.


Graph 1. A. solani from potato
A
Width
Length
10
20
30
40
50
60
70
80
90
100
110
120
130μ
Graph 2. A. solani from Datura
A
B
D
E
F
H
I
J
10
30μ
100μ
10μ
Graph 3. A. brassicae var. nigrescens
B
D
E
F
H
10
20
30
40
50
60
70
80
90
100
110
120μ
U. OF I. S. S. FORM 3

sicae var. *microspora* (Berk.), Sacc., isolated from cabbage leaf spot (*Brassica oleracea* L.); *A. fasciculata* (C. & E.) J. & G., isolated from potato leaf spot; *A. tenuis* Nees, isolated from decaying wood; *A. dianthi* Stev. & Hall, isolated from Dianthus leaf spot; *A. iridicola* (E. & E.) n. com. (*Macrosporium iridicolum* E. & E.), isolated from iris leaf spot (*Iris germanica* L.); *A. sonchi* Davis, isolated from leaf spot of *Sonchus asper* (L.) Hill; *Macrosporium sarcinaeforme* Cavara, isolated from clover leaf spot (*Trifolium pratense* L.); *M. sarcinula* Berk., isolated from decayed spot on Jonathan apple (*Pyrus malus* L.).

All studies were made with pure cultures originating from a single spore of each species used, the spores being located on thin poured plates and transferred before germinating to other plates. The instruments invented by Keitt[1] greatly facilitated this operation.

Whenever it was possible to do so, ten-day-old cultures were used as the standard for comparisons. Measurements of spores and conidiophores were made whenever present. In measuring spores a mechanical stage was used and every spore on the slide which came within the field was measured for both length and width, thus eliminating unconscious selection. Curves of the measurements of each hundred spores were made for each culture. Since in a given species there is frequent variation in the length of the beaks, from one third to six or seven times the length of the spores, the beaks were not in any case included in the measurements.

Graphs giving the superimposed curves of the measurements of one hundred spores of the same species under different conditions were prepared for the sake of easy comparison of the variations due to differences in cultural conditions (Graphs 1–9).

Host Relation and Parasitism

Inoculations were made on the natural hosts, on their near relatives, and on other plants, when there seemed to be any advantage in so doing. For example, *Alternaria solani*, which is morphologically similar to *A. brassicae*, was inoculated on cabbage leaves.

The plants were inoculated by needle pricks and by placing spores in drops of water on the unbroken leaf surface. The percentage of successful inoculations was estimated from the needle pricks, since these were more easily located. An inoculation was considered successful if the fungus to a notable degree invaded and killed the tissues

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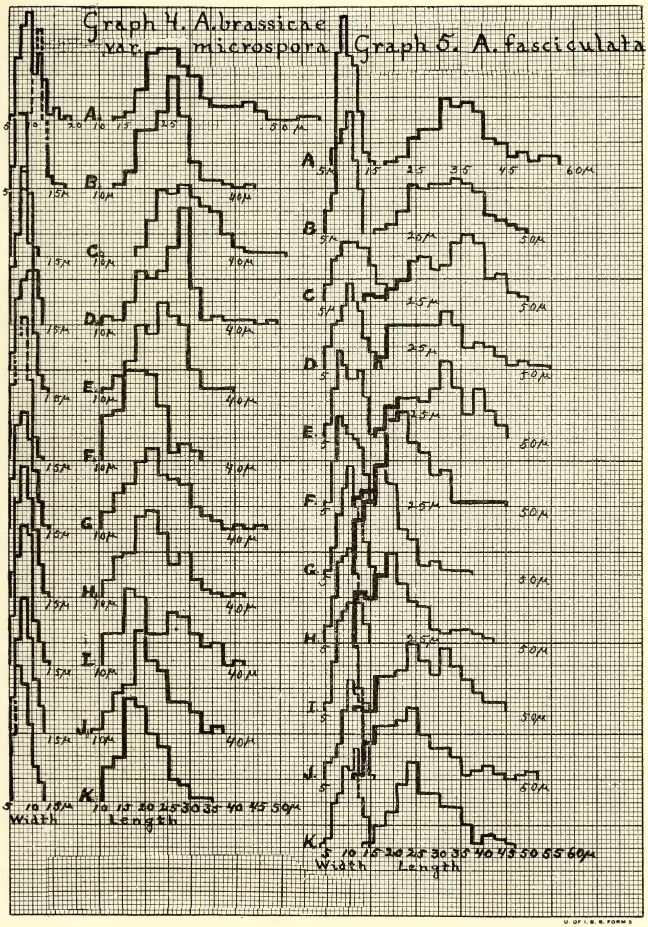
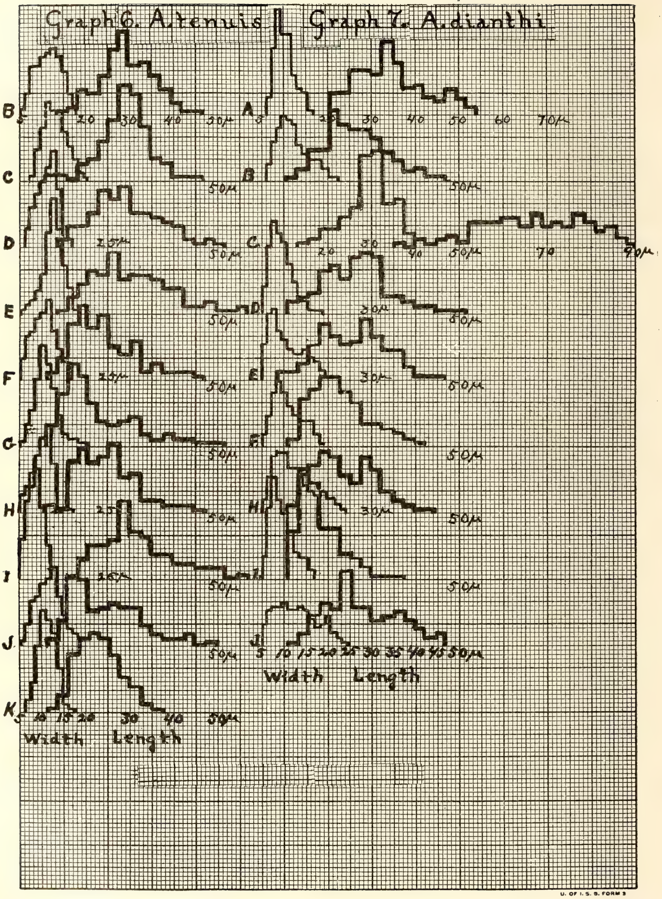
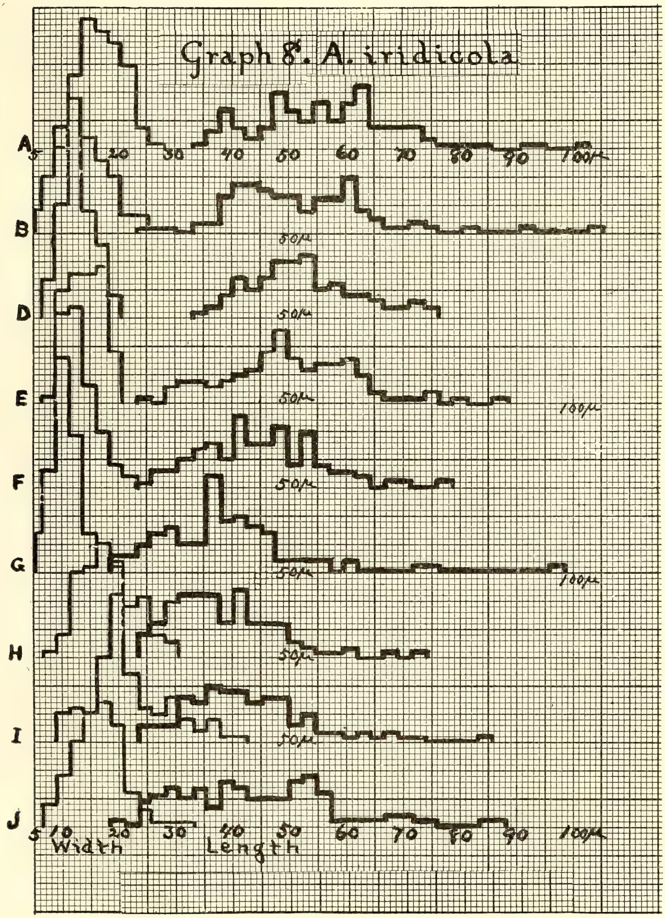
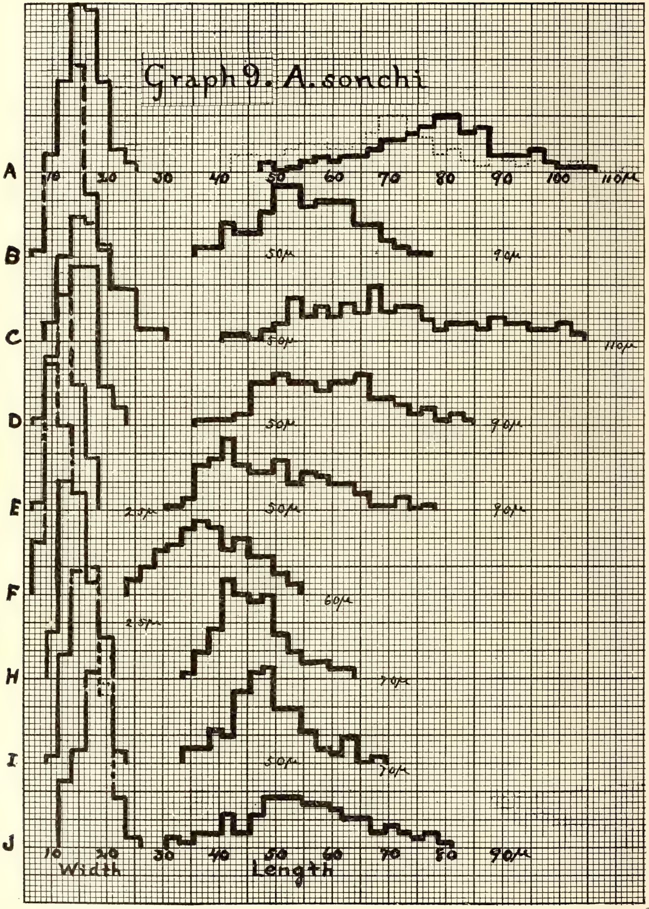
[1] Phytopathology **5**: 266, 1915.


Graph 4. A. brassicae var. microspora
Graph 5. A. fasciculata
A
B
C
D
E
F
G
H
I
J
K
5
10
15
20
25
30
35
40
45
50
55
60
15µ
10µ
40µ
50µ
60µ
25µ
5µ
5 10 15µ
Width
10 15 20 25 30 35 40 45 50µ
Length
5 10 15 20 25 30 35 40 45 50 55 60µ
Width
Length
U. OF I. S. S. FORM 3


Graph 6. A. tenuis
Graph 7. A. dianthi
B
C
D
E
F
G
H
I
J
K
A
B
C
D
E
F
H
I
J
5
20
30
40
50μ
60
70μ
90μ
25μ
30μ
5 10 15 20 30 40 50μ
5 10 15 20 25 30 35 40 45 50μ
Width
Length
Width
Length
U. OF I. S. B. FORM 3


Graph 8. A. iridicola
A
5
20
30
40
50
60
70
80
90
100μ
B
50μ
D
50μ
E
50μ
100μ
F
50μ
G
50μ
100μ
H
50μ
I
50μ
J
5
10
20
30
40
50
60
70
80
90
100μ
Width
Length


Graph 9. A. sonchi
A
10
20
30
40
50
60
70
80
90
100
110μ
B
50μ
90μ
C
50μ
110μ
D
50μ
90μ
E
25μ
50μ
90μ
F
60μ
25μ
H
70μ
I
50μ
70μ
J
10
20
30
40
50
60
70
80
90μ
Width
Length

surrounding the punctures. Unless otherwise stated, successful inoculations also occurred on the unbroken leaf surface. To check the results of the needle-prick inoculations, sterile needle pricks were always made and all the plants kept under identical conditions. In no case did the control plants show signs of fungous invasion around the needle pricks. They will receive no further mention. Unless otherwise stated, spores of the fungus used were always re-isolated from the spots caused by the inoculations. In the following discussion the terms "normal" and "characteristic" indicate that the spot or fungus appeared the same as in the cases of natural infection.

Alternaria solani from potato leaves: Inoculations were made from the first and tenth generations on agar and from spores taken directly from potato leaf spots. The mycelium was used as the inoculum, since very few spores were produced in culture. All of the inoculations with mycelium were made by needle pricks on plants under bell jars. The plants inoculated were: potato (*Solanum tuberosum*); *Datura stramonium;* tomato (*Lycopersicum esculentum* [L.] Mill.) leaves and fruit; *Solanum nigrum* L.; cabbage (*Brassicae oleracea* L.); iris (*Iris germanica* L.); *Lactuca canadensis* L.

All inoculations on solanaceous hosts were successful; about 50 percent of the inoculations on cabbage leaves failed; none succeeded on iris or Lactuca. The development of the spots was less rapid on Datura than on any other of the solanaceous hosts. Spots were all of the characteristic "target board" type. Very few spores were produced on any of the spots—none on the cabbage leaf.

A. solani from Datura leaves: Inoculations by needle pricks and on the uninjured leaf surface were made with spores taken directly from leaf spots and with spores of the first and tenth generations from agar plates. All plants were kept under bell jars. The same plants were used as in inoculations with the strain from potato, and the results were similar except that all attempted inoculations on cabbage failed. The fungus developed least vigorously on potato leaves. On the other solanaceous hosts the spots were not different from those caused by *A. solani* from potato. Very few spores were produced on any of the spots.

A. brassicae var. *nigrescens* from cantaloupe leaves: Inoculations were made with small pieces of diseased tissue, also with spores from the first, sixth and tenth generations on agar, by means of needle pricks and on the uninjured leaf surface. The plants were kept under bell jars for the first few days following inoculations. Inoculations were attempted on cantaloupe (*Cucumis melo*), cabbage, Datura, and Lactuca. Characteristic spots were produced on cantaloupe in all cases; 25 percent of the inoculations on cabbage were successful; no spots were formed on Lactuca or Datura.

The spots produced on cantaloupe leaves, appearing in 2 or 3 days, developed rapidly. The spots on cabbage leaves were mere dots where the spores had germinated on the unbroken leaf surface, but of considerable size where punctures were made. No spores were produced on cabbage and very few on cantaloupe leaves.

A. brassicae var. *microspora* from cabbage leaf spots: All inoculations were made with spores from pure cultures, by needle pricks or on the unbroken leaf surface. Inoculations were attempted on cabbage, radish (*Raphanus sativus* L.), *Lobularia maritima* (L.) Desv., Dianthus, potato, Datura, tomato leaves and fruit. On all cruciferous plants the fungus produced characteristic concentric spots with dark rings of spores on the surface. Spores spread the fungus to other parts of susceptible plants until they were entirely destroyed. On ripe tomatoes dark spots were formed with narrow sharply defined zones of spores. No spots were formed on the other plants used.

A. fasciculata from potato leaf spots: Inoculations were made with spores by means of needle pricks and on the unbroken leaf surface. The plants inoculated were: potato, tomato leaves and fruit, cabbage, radish, Dianthus.

The only definite spots of parasitic appearance were from needle pricks on etiolated or partially etiolated cabbage leaves. The spores and conidiophores on the cabbage leaf spots were very light amber in color, instead of dark olive as is normal. In ripe tomatoes a hard black core was formed in the interior as the mycelium invaded the tissues. This was surrounded by a soft decayed area. The inoculations failed on the other plants.

A. tenuis from decaying wood: Only one series of inoculations was attempted with this species. This was made on Dianthus leaves by means of needle pricks. No spots were formed. The inoculations were made on Dianthus because *A. tenuis* spores cannot be distinguished from those of *A. dianthi*.

A. dianthi from Dianthus leaf spots: Inoculations were made both by needle pricks and on unwounded leaf surface with spores of the first and fifth generations from agar plates. Dianthus and cabbage were the only plants used. No spots were formed on cabbage leaves. All inoculations on Dianthus were successful. In air of natural humidity the spots remained very small but under a bell jar they spread rapidly, produced concentric rings of dark spores on the surface and finally killed the plants.

A. iridicola from iris leaf spots: Inoculations were made entirely by means of needle pricks. Both spores and mycelium were used' The plants inoculated were: Iris, *Lilium philadelphicum* L., Datura, potato, cabbage, Lactuca, cantaloupe, and onion (*Allium cepa* L.). All inoculations except those on iris and onion failed. Spots on iris extended slowly, on onion tops more rapidly, accompanied by the production of a considerable number of spores. Inoculations were made on Datura, potato, cabbage, and cantaloupe because of the general similarity of the spores of *A. iridicola* and *A. solani* (Plate XX, Fig. 7).

A. sonchi from leaf spots on *Sonchus asper:* The fungus could not be isolated from the spots on *Sonchus asper* since the spores would not germinate in culture. Crude inoculations were made on *Lactuca canadensis*, using as the inoculum diseased leaf fragments of *S. asper.* These inoculations were successful and the production of spores on *L. canadensis* abundant. Pure cultures were obtained from *L. canadensis* leaf spots. Inoculations were made by needle pricks and on the unbroken leaf surface. Inoculations were made on *Lactuca canadensis*, *L. sativa* L., *Taraxacum officinale* Weber, cabbage, tomato, and Datura. Spots were formed only on the Compositae. On *L. canadensis* very rapidly spreading characteristic dark brown spots were formed in two days. On cultivated lettuce and dandelion, distinct spots were formed around needle-prick inoculations, but soon ceased to grow and no spores were produced. Spore production was abundant on *L. canadensis.* (Plate XX, Fig. 8).

Macrosporium sarcinaeforme from leaf spots on red clover: Inoculations were made with spores, both on the unwounded leaf surface and by needle pricks. Red clover (*Trifolium pratense*), white clover (*T. repens* L.), alfalfa (*Medicago sativa* L.), cow pea (*Vigna sinensis* Endl.), *Cucumis melo*, and Allium, were inoculated. The fungus was actively parasitic only on the clovers and alfalfa. Spots developed most rapidly on red clover and spores scattered the disease over the entire plant. On white clover and alfalfa the spots did not spread to leaves that had not been inoculated. In all cases the spots appeared within three days of inoculation. On onion, small white spots were occasionally produced around needle pricks but no spores were produced and the parasitism was evidently very feeble.

M. sarcinula from apple fruit spot: This fungus was isolated by Miss Jean MacInnes, of the University of Illinois, in her studies of the rots of apples.[3] It appeared to be causing an apple rot. Small black pycnidia-like bodies were scattered frequently over the rotted area under the epidermis of the apple. These also appeared in the first generation on agar. When broken they appeared to be merely sclerotia. Spores from pure cultures were used in making inoculations both by needle pricks and on uninjured leaf surface. The fungus conformed to descriptions and figures of *M. sarcinula.* The same plants were inoculated with *M. sarcinula* as with *M. sarcinaeforme.* Spots were produced only on red clover and alfalfa. All attempts to inoculate apples failed. The spots on red clover were lighter colored but otherwise almost identical with those caused by *M. sarciniaeforme.* Few spores were produced on the spots. On the onion tops the fungus produced small white spots surrounding the punctures but no spores were formed.

The most noteworthy results of the inoculation experiments occurred in respect to *A. solani, A. sonchi, A. brassicae* var. *nigrescens,* and *M. sarcinula.* Both strains of *A. solani* grew freely on all of the solanaceous hosts, and one strain was feebly parasitic on cabbage. Morphologically, judging from exsiccati, *A. solani* and *A. brassicae* are identical, and the inoculation experiments might be considered further evidence of this view. *A. sonchi* was actively parasitic on Lactuca, a near relative of Sonchus, and was possibly very feebly parasitic on other Compositae. *A. brassicae* var. *nigrescens* proved slightly parasitic on cabbage although this would hardly be expected from nearness of Cruciferae and Cucurbitaceae. *M. sarcinaeforme* and *M. sarcinula* are entirely dissimilar except in general form of spores, yet they appeared equally parasitic on red clover and alfalfa, and both were feebly parasitic on onion tops.

Cultures on Agar

For purposes of isolation and general study, standard lima bean agar (beans 100 g., agar 15 g., water 1,000 g.) was used. The cultures were kept in darkness at 30° C., with the exception that *A. sonchi,* which would not grow at 30°, was grown at 20°. In testing the effect of a lower temperature, cultures on standard bean agar were kept at 10° C. The effect of acidity, also alkalinity, was determined on standard bean agar, using 30 cc. and 20 cc. of normal hydrochloric acid, or 20 cc. of normal sodium carbonate per liter. A standard synthetic agar was used to study the effect of nutrition: 1.36 g. acid potassium phosphate, 1.06 g. sodium carbonate, .5 g. magnesium sulphate, 5 g. glucose, 1 g. asparagin, 15 g. agar, 1000 g. water. Variations from this standard were made by omitting glucose, doubling the amount of glucose, omitting asparagin, and by doubling the amount of asparagin. Plain agar, 15 g. per liter of water, washed for several days in distilled water, was also used.

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[3] Unpublished thesis.

Records of all cultures on the various media were made in tabular form, but for the sake of brevity only the table for cultures on bean agar is given here; the differences shown on other media being briefly summarized. Variations in size of spores are given on graphs one to nine.

The most striking characters brought out by the colonies on the standard bean agar were the wide differences in the two strains of *A. solani* which on their hosts are indistinguishable. The strain from potato produced a pure white colony with marked red chromogenesis in the medium, had straight colorless submerged mycelium and no spores. On account of the abundant production of conidiophores and spores the strain of *A. solani* from Datura formed a gray colony, it produced no chromogenesis, and the submerged mycelium was dark olive and torulose. *A. fasciculata*, *A. tenuis*, and *A. dianthi* produced spores indistinguishable from each other. The conidiophores of *A. dianthi* were slightly larger in cross section than those of the other two. In general appearance of the colonies and in the production of aerial mycelium these three species were different. The other Alternaria species studied were quite distinct in most cultural characters. The two species of Macrosporium were totally unlike except in general form of spores.

Bean agar, 10°, 30 days: Zonation was absent or inconspicuous in most of the colonies, especially in those of *A. brassicae* var. *microspora* which at 30° had the most marked zonation of all the species. This species also showed a marked alteration in color and form of its spores, these being light amber, nearly colorless, and about half the width of the normal dark olive spores. Another marked change occurred in the size and color of the spores of *A. dianthi*, these being twice their normal

TABLE B.

Fungus	Growth, Mm.	Zonation General Character	Aerial Mycelium	Submerged Mycelium	Conidiophores	Spores
Alternaria solani from potato	33	Pure white, wide zoned, medium colored brick red.	Abundant, long white, cottony.	Colorless, straight.	None	None
A. solani from Datura	33–35	Gray, wide zoned colony, no chromogenesis.	Abundant, long, white.	Dark olive, much branched and torulose.	Abundant, normal or much constricted.	Abundant, normal in size, but much constricted.
A. brassicae, V. nigrescens	42	Very dark, especially near the center	Very little, white.	Dark olive, straight.	Few, dark olive, normal.	Few, dark olive, normal.
A. brassicae, V. microspora	28–30	Black colony with narrow sharply defined zonation.	Very little, short, white, weak.	Colorless or nearly so, straight.	Abundant, light-olive, normal.	Very abundant, normal.
A. fasciculata	32	Gray, wide zoned.	Abundant, white, long.	Colorless, turning olive with age, straight.	Abundant, light olive.	Very abundant, normal.
A. tenuis	27	Dark in center, pale on outer edge, zonation well defined.	Little, white, long.	Colorless at first, turning olive, straight.	Not abundant, light olive.	Fairly abundant, normal.
A. dianthi	15–16	Gray, zoned colony.	Considerable, long, white.	Colorless, or very light olive, straight.	Abundant, light olive.	Abundant, normal.
A. iridicola	15–17	White or gray cottony, zonation ill defined.	Long, white, cottony.	Colorless, straight.	Fairly abundant, very pale olive.	Fairly abundant, normal, light olive.
A. sonchi	25, 20° C.	Pure white, cottony colony with dense mycelial zone, slight yellow chromogenesis.	Very abundant, white, cottony.	Colorless, straight.	In clumps, very pale olive, normal.	In clumps under mycelial zones, smaller than normal.
Macrosporium sarcinaeforme	10–12	Black, dense colony.	Very little, short, white, weak.	Dark olive, much branched and torulose	Abundant, dark, normal.	Abundant, dark olive, normal.
M. sarcinula	10–13	Cottony, white.	Abundant, long, white, cottony.	Colorless, straight.	Abundant, light olive, normal.	Abundant, olive, normal.

dimensions and of much lighter color (Plate XIX, 10). A marked change in the character of both the aerial and submerged mycelium occurred in *M. sarcinaeforme*, which is normally black, but here was white. All colonies were much retarded in rate of growth, the amount varying with the species. Five produced no spores.

Bean agar + 30 Fuller's scale, 30°, 10 days: This medium did not solidify and in many cases the whole colony was saturated with it. There was little variation in the general appearance of most of the colonies from that on standard bean agar. Two variations worthy of note were the absence of zonation in colonies of *A. brassicae* var. *microspora*, and the more than usual production of aerial mycelium on colonies of *A. brassicae* var. *nigrescens*. The chromogenesis of *A. solani* from potato was more marked on this than on any other medium. Some species which usually produced spores in more or less abundance produced few spores or none on this medium. They were *A. brassicae* var. *nigrescens*, *M. sarcinaeforme* and *M. sarcinula*. The spores of *A. sonchi* and *M. sarcinula* were below normal size.

Bean agar + 20 Fuller's scale, 30°, 10 days: Except for a little greater production of aerial mycelium by most species, the general appearance of the colonies was like those on neutral bean agar. Chromogenesis of *A. solani* was very marked, also the submerged mycelium of this species was darker than normal. *A. brassicae* var. *nigrescens*, *M. sarcinaeforme*, and *M. sarcinula* produced fewer spores than on neutral agar.

Bean agar − 20 Fuller's scale, 30°, 10 days: On the alkaline bean agar the most notable feature was the abundant production of spores by *A. brassicae* var. *nigrescens*, which ordinarily produced few spores on artificial media or on its natural host. In this instance the spores covered all parts of the colony. *A. solani* from potato, which on neutral and acid media produced marked chromogenesis, on the alkaline medium produced none, and the submerged mycelium, in the older part of the colonies, became dark. *A. dianthi* and *M. sarcinula* produced spores smaller than normal on the alkaline medium. The general appearance of the colonies was little different from that on neutral bean agar with the exceptions already noted.

Standard synthetic medium, 30°, 10 days: Except for a general lack of aerial mycelium and the appearance of less luxuriance, the colonies were little different from those on the standard lima bean agar.

Standard synthetic agar minus glucose, 30°, 10 days: The appearance of the colonies on this medium was very similar to that on washed plain agar, although the appearance of starvation was not so emphasized.

Standard synthetic agar with double amount of glucose, 30°, 10 days: The aerial parts of the colonies, mycelium, conidiophores and spores, were more abundant than on the standard medium. The size of the spores of some species was much reduced (Graphs 4, 8, 9; Curve H).

Standard synthetic agar with double amount of asparagin, 30°, 10 days: All colonies appeared less luxuriant than on the standard synthetic agar or bean agar.

Standard synthetic agar minus asparagin, 30°, 10 days: Partial starvation appeared in all species, and the submerged mycelium was darkened in most cases. The latter was most noticeable in *A. solani* from potato which usually had colorless mycelium. This same fungus produced a few spores on this medium, which was also unusual. Aerial mycelium was generally very meager in all species.

Plain washed agar, 20°: But for the mycelium of *Macrosporium sarcinaeforme*, the only characters by which the different species could be identified were those of the spores and conidiophores which were natural (Plate XIX., 1, 3, 5, 7, 8, 11, 16, 17; XX: 7, 9). Aerial mycelium was lacking and the submerged mycelium colorless and without distinctive features. Spores and conidiophores were characteristic. This was the only artificial medium upon which *A. solani* from potato produced spores in any abundance. *A. solani*, from Datura, did not produce spores until after eighty days. The torulose mycelium characteristic of this strain on most media was scarcely noticeable on the washed agar. *Macrosporium sarcinaeforme* produced dark torulose submerged mycelium characteristic of the species.

Variations under Different Conditions of Humidity

The effect of humidity upon the colony characters was studied by keeping the cultures at 20° C. in saturated atmosphere; room humidity (variation 45–70 percent); and in an atmosphere kept dry by the use of calcium chloride. In order to prevent desiccation of the medium, all the plates were coated with a thin layer of paraffin. After the agar had hardened, hot sterile paraffin was poured over the agar and the excess poured off. This left a thin film of paraffin effectively protecting the medium from desiccation or contamination. The paraffin was punctured in several places by needle pricks and spores of the fungi planted in one of the punctures. The cultures were kept with the plates inverted, without covers. In no case was there any contamination after the plates had been coated and very little occurred during the process. In the least humid atmosphere there appeared to be no change in the water content of the medium even after sixty days.

In all cases where aerial mycelium was produced, there was an increase in its production in the saturated atmosphere over that under ordinary conditions. Those species producing the greatest abundance of mycelium produced proportionately fewer spores. On account of the presence of the paraffin film, the usual colony form was lacking. *A. brassicae* var. *microspora* and *M. sarcinaeforme*, both of which produced almost no aerial mycelium, produced an abundance of spores. At room humidity little mycelium was produced, and that was in tufts around the needle pricks. The relative production of spores was greater than in the saturated atmosphere; the submerged mycelium grew rapidly under the paraffin and covered the plate. In some cases the conidiophores ruptured the paraffin coat. This was true of *M. sarcinaeforme* to a greater extent than of any other species. In the very dry atmosphere no aerial mycelium and no spores were produced with the exception that *M. sarcinaeforme* produced a few spores in air bubbles in the paraffin coating.

Reaction to Bacterial Influence

One species of bacterium which will be designated as *B. x*, occasionally occurred as a contamination on plates. It had a marked inhibitory action upon the growth of the fungi and was therefore used for special study. Plantings of all the species of fungi that were being studied were made in the usual way, then after four days the plates were inoculated on four sides at about 1 mm. from the outer edge of the colonies with cultures of *B. coli*, 2 strains, *B. subtilis*, and *B. x*.

In all cases the fungi grew through the colonies of *B. coli* without showing any reaction. Occasionally in colonies of *B. subtilis* there was slight darkening of the mycelium. On the sides of the fungous colonies opposed to the colonies of *B. x*, however, there was in every case a marked reaction by the submerged mycelium. The hyphae were inhibited greatly in growth, were much darkened and extremely torulose. Nodules were produced along the hyphal threads and at the extremities of hyphal branches. The reaction by all the fungi was essentially the same. In all cases there was no resemblance between the normal and the abnormal mycelium (Plate XX: figs. 1–6). The aerial parts of the colonies did not show reaction to the bacterial influence.

CHROMOGENSIS ON RICE

On account of the chromogenesis exhibited by two of the species, cultures were made on boiled rice (1 g. rice, 10 g. water autoclaved at 120° C. in test tubes). The colonies were allowed to grow for two months before comparisons were made, although it was noted that *A. solani* from potato, *A. sonchi* and *M. sarcinula* showed more or less marked chromogenesis within three or four days after inoculation.

The most marked variations were those between the two strains of *A. solani*, which throughout the other experiments had shown many differences in reaction to media. *A. solani* from potato colored the rice light orange-red at the bottom of the test tube, deep orange-red or brick-red at the lower limit of mycelial growth. The part of the rice through which the mycelium grew was dark-red-brown. The aerial mycelium was also red-brown. *A. solani* from Datura did not color the medium below the mycelium. The part of the medium occupied by the colony was dark gray or black. The aerial mycelium was white. *A sonchi* colored the medium lemon-yellow; *A. iridicola*, yellow-brown; *A. fasciculata*, *A. tenuis*, and *A. dianthi* light brown; *A. brassicae* var. *nigrescens* gave a very faint brown; *A. brassicae* var. *microspora* gave no coloration. The medium was colored deep pink by *Macrosporium sarcinaeforme;* blue-gray by *M. sarcinula*.

The remarkable thing about the reactions of these fungi to various media was that it was impossible to predict from the reaction of one species, what the reaction of another would be. A case in point was the reaction to alkaline and acid media. *A. brassicae* var. *nigrescens* showed a gradual increase in production of spores from high acid to alkaline media, the production in the latter case being very abundant. *A. solani*, from Datura, produced very few spores on the alkaline medium while on the acid media the spore production was abundant. Several species showed a lowered spore production in the acid media, notably those which normally produced few spores. *Macrosporium sarcinaeforme*, which usually produced spores in great abundance, produced none on the higher acid medium.

Lack of nutritive substances affected all species alike, causing a general lack of luxuriance, lack of color in submerged mycelium and absence of aerial mycelium. This reached the extreme on the leached agar, where the aerial mycelium was almost entirely lacking and the submerged mycelium colorless and not at all characteristic, with the exception of *M. sarcinaeforme*.

The comparative studies noted above were made with cultures which were ten days old, but all of the cultures were maintained thirty days or longer—sometimes ninety days. In some cases marked variations due to age were noted in the spores. In several species secondary growth or development of spores occurred which entirely destroyed their distinctive characters. This secondary development consisted in a multiplication in the number as well as a great enlargement of the cells. This caused the spores to become very irregular in form, much darker, deeply constricted, and very much larger than normal (Plate XIX: 2, 6, 10).

As a result of the secondary development, the spores of Alternaria assumed the form of Mystrosporium, as was pointed out by Constantin (4). Corda (6) in his description of the genus Mystrosporium made figures which are undoubtedly of Alternaria spores in an advanced stage of secondary growth. Later (6) he figured the development of *M. stemphylium* Corda from the regular form of a young Alternaria spore through the various stages of secondary development to the very irregular form called Mystrosporium. From the descriptions of species of Mystrosporium there is no doubt that most if not all of them belong to Alternaria. All specimens labeled Mystrosporium which I examined in the exsiccati were Alternaria in various stages of secondary enlargement.

Young colonies of all species of Alternaria produced smooth regular spores which remained regular for a time interval which varied in different species and on different media. The richer the medium the earlier the secondary enlargement began. The first indications of this secondary growth were multiplication of and deep constriction at the septa. On leached agar no secondary enlargement occurred in any species, even after a lapse of three or four months. There occurred irregularly in all species a well-marked echinulation of the spores. Beyond a deep constriction at the septa, no secondary change was noted in Macrosporium, nor in *A. sonchi*, *A. brassicae* var. *microspora*, and *A. solani* from potato. This last, however, produced spores in numbers sufficient for observation only on leached agar where secondary enlargement did not occur in any species.

V. DISCUSSION

A general survey of the characters upon which specific descriptions of Alternaria and Macrosporium are, or may be, based is fitting in this place.

Broadly considered, the different bases for descriptions may be divided into natural morphology, cultural morphology and media changes, and host relations. In the present studies effort was made to get a fair estimate of the value of each of these by studying them under various conditions. In specific descriptions the shape, size, and color of spores are generally given. Also septation, constriction, and echinulation are more or less frequently mentioned. In many instances one of these characters may furnish the only point of differentiation of one species from another species in the description of which that character is not mentioned. The conidiophores are generally more or less definitely described, the characters commonly given being, length, width, septation, constriction, color, branching and geniculation. As in the case of the spore characters, any one or all of these characters may be neglected by one author or made the basis for specific description by another. The nature of the submerged mycelium has been made the basis for specific descriptions in some cases, although in many cases it is not mentioned. In artificial cultures where the mycelium is easily studied it is generally taken into consideration. The characters noted are thickness, length between septations, color, constriction, fasciculation and rate of growth. Aerial mycelium is not often mentioned in specific descriptions. Length, width, color, abundance, and general habit are the characters considered.

MORPHOLOGY

1. Spore: The previously described experiments show that, within the natural range, the size of the spores under natural conditions was, in almost all of the species studied, quite constant, although under ordinary cultural conditions the size of the spore on artificial media is usually less than that on the natural host. However, in a relatively short time—two to six weeks—in some species there is a marked secondary development.

The most characteristic thing about the spores of these fungi is their shape which is an immediate index to the genera, and generally to the species. All obclavate, cuneate, ovate, pointed or beaked spores belong to Alternaria and under suitable conditions form chains. There is variation in shape within a single species, but in most cases under natural conditions the shape of the spore, combined with its size, identifies the species. Parasites, as they are collected during the period of active attack upon plants, are uniform in spore form. Some species wintering on decaying leaves showed a secondary development which quite destroyed their characteristic form and size and made recognition impossible. On artificial media where secondary growth occurred frequently, the shape of the spores was greatly changed.

All Alternaria spores have a more or less acute apex and some species have beaks. In some cases the beaks are six or seven times the length of the spore itself. This, however, occurs only when they are not borne in chains, or in the terminal one of a chain. In the chains the terminal spore is formed by budding out of the apex, or occasionally from the side, so that the spore above takes the place of the beak of the spore below. For this reason the length of beak alone cannot be taken as of great value. Many species of Alternaria do not have beaked spores, so to a certain extent the beak is a specific character.

There is marked difference between species in the number of septa. Septation is dependent on the size of the spore in a single species, but independent of the size of spore in different species. The amount of septation increases with the age of the spore. The same conditions which brought about changes in shape and size of spores, increased the number of septa and constriction at the septa.

Echinulation occurred at times in all of the species studied. In some cases part of the spores from a colony were echinulate while others were smooth.

When the age of the spore is considered, color is an important character of some species. Generally spores darken with age. *Alternaria dianthi* and *A. brassicae* var. *microspora* grown at 10° showed marked color change, both being very light amber instead of the normal dark olive. *A. fasciculata*, forming spots on partially etiolated cabbage leaves and also in growing on raw disinfected cabbage leaves and petioles, showed a similar change.

2. Conidiophore: The form of the conidiophore is often quite characteristic, although length depends upon the age and environment. In *Macrosporium sarcinaeforme* the end of the conidiophore becomes swollen before the spore appears. If this spore falls off another bud forms at the end of the conidiophore, producing another "joint" which in turn swells at the end and produces a spore. If the spore

does not fall off, the conidiophore may bud again at the side of the swollen end and produce a second short "joint" which bears a second spore. This process, repeated, causes the geniculation which is sometimes given as a specific character. Branching occurred occasionally in all the species but was not often observed under natural conditions. The width of the conidiophores was quite constant. Constriction differs between species and with the age of the conidiophore. In some species no constriction was noted while in others the constriction was marked.

Color is as distinctive of the conidiophores as of the spores and is usually concolorous with that of the spores. The only variations in the color of conidiophores on artificial media corresponded with the color change of spores.

3. Aerial mycelium: Parasitic species under usual conditions produce little or no aerial mycelium. Under humid conditions, a very little aerial mycelium was sometimes produced by some species. This was always lax and white, and although in some species it was thicker in cross section than in others, it was so nearly featureless as to be thought worthless in classification. More or less aerial mycelium was produced by all species under some conditions of artificial culture. In some species, notably *A. brassicae* var. *microspora* and *M. sarcinaeforme*, it could be found only with the microscope. Other species produced aerial mycelium in abundance under humid conditions on rich media.

4. Submerged mycelium: The characters of the submerged mycelium were the most variable of all characters studied. Under ordinary cultural conditions on standard media the mycelium of some species had distinguishing characteristics, but any change in the cultural conditions might bring about a change in the characters of the mycelium which would make its identity doubtful. On low-grade nutrient agar the mycelium of most species was colorless and, with a few exceptions, characterless. On some media the color, width, and general character might separate certain species from certain other species, but under the influence of strong inhibition, such as was brought about by bacterial influence, the changes entirely destroyed any distinguishing characters. In most cases age greatly affected the mycelium in color, septation, and constriction.

Host Relation

Inoculations upon host plants show that some species are quite narrow in their range; *e. g.*, *A. sonchi* was parasitic on *Sonchus asper* and *Lactuca canadensis* but failed to maintain itself on cultivated lettuce and other Compositae. Others may be broader in their range, growing actively on many species of one family and less actively on other families; as, *A. solani* which grew actively on all solanaceous hosts used and less actively on cabbage. *A. brassicae* var. *microspora* grew actively on all cruciferous hosts inoculated but failed in all inoculations outside of that family. Forms morphologically inseparable may grow on different hosts and fail to cross-inoculate, or of several forms morphologically alike only one strain may be parasitic. On the other hand, forms which are morphologically clearly distinct may be equally parasitic on the same host.

The studies here presented would seem to show that the present tendency toward limiting the distinction of species to differences in morphology, regardless of host relationships, is commendable. They would seem to indicate, further, that characters should be sought which are least affected by cultural conditions, and in using such characters due consideration should be taken of factors liable to make those characters variable; such as, age or stages in development.

VI. GENERIC LIMITATIONS OF ALTERNARIA AND MACROSPORIUM

A. Alternaria

In every case where species of Alternaria have been described, the spores have been more or less elongated and pointed at one end. In the present studies all spores of the obclavate, cuneate, or ovate form produced chains of spores under favorable conditions. Since the species studied in culture covered all the types of spores found in exsiccati or described in the "Sylloge Fungorum" under the genera Alternaria and Macrosporium, there is no doubt that all of the species with these types of spores belong to the genus Alternaria, that most of the species named under Macrosporium belong to this genus, and can be recognized as such by the descriptions given of their spores.

The term "clavate," as applied to the Alternaria spores, is a misnomer arising from a misconception and carrying misconception with it. Without exception the pointed end of the spore is the apex and the rounded end is the base. *Alternaria tenuis* has been figured with its spores attached by their apices, and as this error was widely copied it undoubtedly went far toward spreading the misconception. Other species of Alternaria, described as Macrosporium, have been similarly figured and it is a common conception that the beak is a pedicel. Many authors have made correct descriptions and figures Alternaria. In such cases the term "obclavate" replaces "clavate."

The generic description of Alternaria[4] should be emended as follows:

ALTERNARIA Nees. Conidiophores solitary or fasciculate, erect or subdecumbent, simple or branched, generally short, colored. Conidia muriform, often with few longitudinal septa, ovate, obclavate, or elongate, always with more or less definitely pointed apex, often long-beaked, colored; under favorable conditions forming chains. (Ex., *A. tenuis*, the type of the genus.)

B. MACROSPORIUM

Of the four species of Macrosporium described by Fries when he created the genus, *M. convallariae* and *M. cheiranthi* undoubtedly belonged to Alternaria. Fries, however, had rejected the genus Alternaria and placed *A. tenuis* in the genus Torula as *T. alternata*. In the exsiccati studied, specimens labeled as *M. convallariae* and *M. cheiranthi* belonged to Alternaria. The third species of Fries, *M. tenuissimum* (*Helminthosporium tenuissimum* Nees), is placed by Saccardo in the genus Clasterosporium as *C. tenuissimum*. The fourth species of Fries's original publication, *M. caracinum*, is not mentioned by Saccardo. The material for this species was supplied to Fries (7) by Schweinitz, and the description of Fries corresponds exactly with the description and figures of Schweinitz (18) for *Clasterosporium caricinum*, which is the type of the genus Clasterosporium. There is no doubt, therefore, that the types of *Clasterosporium caricinum* and *Macrosporium caricinum* are from the same material.

Corda (6) placed *Clasterosporium caricinum* in the genus Sporadesmium as *S. closteriosporium*, but the genus Sporodesmium was at that time so heterogeneous that such a disposition cannot be considered final.

Since *Macrosporium caricinum* Fries and *M. tenuissimum* (Nees) Fries were published two years before *Clasterosporium caricinum* Schw.,

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[4] ALTERNARIA Nees, Syst. d. Pilze 11, p. 72 (Etym. *alternus* ob conidia alterne crassiora et tenuata). Hyphae fasciculatae, erectiusculae, subsimplices, breves. Conidia clavato-lageniformia septato-muriformia per isthmos (conidiorum caudas) catenulatim digesta, mox vero secedentia.—*Polydesmi*, *Macrosporii* et *Clasterosporii* species nonnullae obiter observatae forte huc spectant. (Saccardo, Sylloge Fungorum IV: p. 545.)

M. tenuissimum (Nees) Fries becomes the type of the genus Macrosporium, which will take over the species now given under the genus Clasterosporium, Clasterosporium being antedated by Macrosporium.

All but twenty of the species of Macrosporium listed in the "Sylloge Fungorum" belong to Alternaria. These twenty are given as having globular and sarcinaeform, or globular and clavate spores. The latter species may possibly have both forms of spores but it is much more probable that in most cases the "clavate" spores belong to Alternaria and that two distinct forms were present when the species were described. Such mixtures were found in the exsiccati in several instances. Of the two species studied in culture *M. sarcinula* very rarely produced a pointed spore bearing another spore and might be considered a bridging species to Alternaria.

All of the twenty species given in the "Sylloge Fungorum" having sarcinaeform or globose spores are one species in so far as spore measurements are dependable. Of the thirteen specimens found in exsiccati, however, there were two distinct forms, and two distinct forms were cultured during the present studies. Some of the names of the twenty species in question have already been reduced to synonymy and it is probable that only a few separable forms exist.

There is nothing in the morphology of the species of this group which would exclude them from the genus Stemphylium.

The species now named Macrosporium which should be put into Stemphylium, providing they are distinct species, are: *M. sarcinula* Berk, *M. sarcinaeforme* Cav., *M. abruptum* C. & E., *M. baccatum* Ell. & Kell., *M. valerianellae* Roum., *M. elegantissimum* Rabh., *M. pelargonii* E. & E., *M. globuliferum* Vestergr., *M. toruloides* E. & E., *M. nodipes* Sacc., *M. schemnitziense* Baumel., *M. puccinioides* E. & And., *M. chartarum* Pk., *M. myrmecophilum* (Fr.) Sacc., *M. nitens* (Fr.) Sacc., *M. subglosum* Cke. & Rav., *M. rosarium* Penz., *M. septosporum* Rabh., *M. atrichum* C. & E., *M. parasiticum* Thum., *M. commune* Rabh.

VII. SPECIFIC LIMITATIONS OF ALTERNARIA

Except for the species named above as belonging to Stemphylium, all the species of Macrosporium in the "Sylloge Fungorum" belong to Alternaria, and can be recognized as Alternaria from their descriptions.

In the present studies enough variation due to age alone occurred in *Alternaria tenuis*, *A. fasciculata*, and *A. dianthi*, to fit these to a large percentage of the species of Alternaria and Macrosporium described in the "Sylloge Fungorum" and found in the exsiccati. No variations occurred in the forms in group *6*, of the exsiccati material studied except those which would occur due to age, and all in this group might be considered *A. tenuis* and varieties of *A. tenuis*. Even the narrowest limitation in the application of specific names would preclude the retention of many of the present species.

No final disposition of the present specific names of Alternaria and Macrosporium can be made without a study of authentic specimens of each species. Most of the descriptions are far from being complete or definite enought to permit their being used for this purpose. For convenience a tentative grouping of similar forms which may be identical, and which are undoubtedly closely allied, should be made. These groups might well be retained to indicate the similarity of a number of forms such as is exemplified in bacteriology in the *B. coli* and *B. subtilis* groups. As in bacteriology, each group should be designated by a typical species.

The groups suggested are as follows:

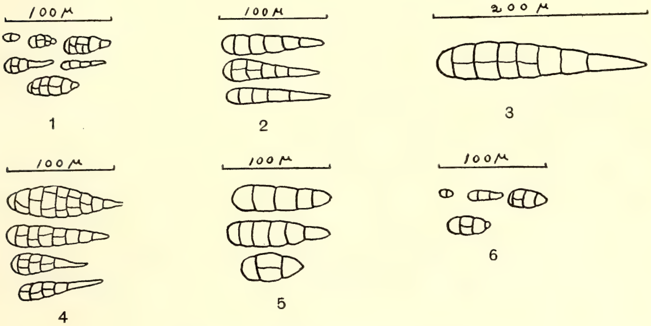
The *A. tenuis* group. This group is characterized by spores ranging from 11–50 × 7–20 μ. The spores are quite variable in form as well as in size but are generally broad and muriform (fig. 1, spores of the *A. tenuis* group). All specimens mentioned in group *6*, page 446, belong here.

The *A. brassicae* group. This group should contain regular, long, tapering, acute-beaked spore forms with measurements ranging from 35–120 × 10–30 μ. The spores have few longitudinal septa and are often long beaked (fig. 2, spores of the *A. brassicae* group). All of group *3*, page 444, belong in this group.

The *A. herculea* group. *A. herculea* (E. & M.) com. nov. (*Macrosporium herculeum* E. & M.) is the type of a group with spores similar in form to those in the *A. brassicae* group but much larger (fig. 3, spores of the *A. herculea* group). To this belong the specimens mentioned in group *4*, page 445.

The *A. cucumerina* group. *A. cucumerina* (E. & E.) com. nov. (*Macrosporium cucumerinum* E. &. E.), syn. *A. brassicae* var. *nigrescens* Peglion, is typical of a group similar in spore form to that of *A. brassicae* but with the spores uniformly wider and more muriform and, generally, shorter (fig. 4, spores of the *A. cucumerina* group). In this group should be placed all in group *5*, page 445.

The *A. sonchi* group. *A. sonchi* Davis is different from any of the species in the above groups in having large-celled spores with a markedly obtuse apex (fig. 5, spores of the *A. sonchi* group). The range in size is 50–125 × 12–25 μ. No similar form was found in exsiccati.



TEXT-FIGS. 1–6.—For explanation see text.

The *A. brassicae* var. *microspora* group. The spores of *A. brassicae* var. *microspora* (Berk.) Sacc. differ from those of *A. tenuis* in being uniformly narrower and less muriform; longitudinal septa seldom being formed (fig. 6, spores of the *A. brassicae* var. *microspora* group). Here belong those of group 7, page 447.

A more complete study of exsiccati might show the necessity of other groups for forms not included above.

Without doubt the number of valid species of Alternaria is a very small percentage of the present named species of Alternaria and Macrosporium, but it is outside of the limits of the present paper to reduce these names to synonymy.

VIII. SUMMARY.

1. Spore shape in the genus Alternaria is correlated with catenulation of spores.
2. All obclavate, ovate, cuneate, or elongate-pointed spores of the Macrosporium-Alternaria type form chains and belong to Alternaria.
3. The acute end of the spore is the apex or beak, not the base or pedicel.

4. Of these spores, all globular, sarcinaeform, cubed, or oblong spores without apex or beak belong to Stemphylium.

5. Macrosporium, by priority, becomes the name of the genus which has been known as Clasterosporium Schw.

6. Of the four species described by Fries when he created the genus Macrosporium, two belonged to Alternaria and *M. tenuissimum* (Nees) Fries becomes the type of the genus Macrosporium.

7. In these genera, conidiophores and conidia possess the only suitable characters upon which to describe species.

8. Conidia in many species go through secondary changes which destroy their distinctive characters.

9. Secondary changes in conidia may be due to age or to abnormal environment.

10. The genus Mystrosporium was described from Alternaria in advanced secondary development.

11. Mycelial characters are too easily affected by external conditions to be dependable in describing species.

12. Slight changes in media may cause great changes in the submerged mycelium.

13. Identical changes of environment may bring about opposite reactions in different species.

14. Morphology of the dependable stable characters under normal conditions is the most useful basis for describing species.

15. Due consideration must always be taken of the age of the organism in describing a species.

16. For convenience, the genus Alternaria should be divided into groups of species having similar spores.

The writer takes this opportunity to express his thanks to Professor F. L. Stevens for encouragement and many helpful suggestions in carrying out this work; to Professor Wm. Trelease for aid in solving the taxonomic problems involved; and to others, mentioned or not mentioned in this paper, who have given assistance in any way.

University of Illinois, Urbana.

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EXPLANATION OF PLATES XIX AND XX

PLATE XIX

All drawings were made with camera lucida to scale shown on the plates.

1. *Alternaria brassicae* var. *nigrescens* on leached agar.

2. *Alternaria brassicae* var. *nigrescens* spores showing stages in secondary development.

3. *A. solani* from Datura on leached sugar.

4. *A. solani* from Datura, spores from bean agar culture.

5. *A. solani* from potato on leached agar.

6. *A tenuis* spores two weeks and six weeks old showing secondary development due to age.

7. *A. tenuis* on leached agar.

8. *A. dianthi* on leached agar.

9. *A. dianthi* spores from colony on acid agar.

10. *A. dianthi* spores from colony on bean agar at 10°, showing enlarged size and secondary development.

11. *A. fasciculata* on leached agar.

12. *A. fasciculata* spores showing echinulation.

13. *A. fasciculata* showing development of a spore.

14. *A. brassicae* var. *microspora* on leached agar.

15. *A. brassicae* var. *microspora* on bean agar, 10°, showing reduced size of spores and lack of color.

16. *Macrosporium sarcinaeforme* on leached agar.

17. *M. sarcinula* on leached agar.

Plate XX

1. Mycelium of *A. solani* from potato; *a*, reaction to bacterial influence; *b*, normal.

2. Mycelium of *A. brassicae* var. *microspora; a* and *b* as in No. 1.

3. Mycelium of *A. brassicae* var. *nigrescens; a* and *b* as in No. 1.

4. Mycelium of *A. solani* from Datura; *a* and *b* as in No. 1.

5. Mycelium of *A. tenuis; a* and *b* as in No. 1.

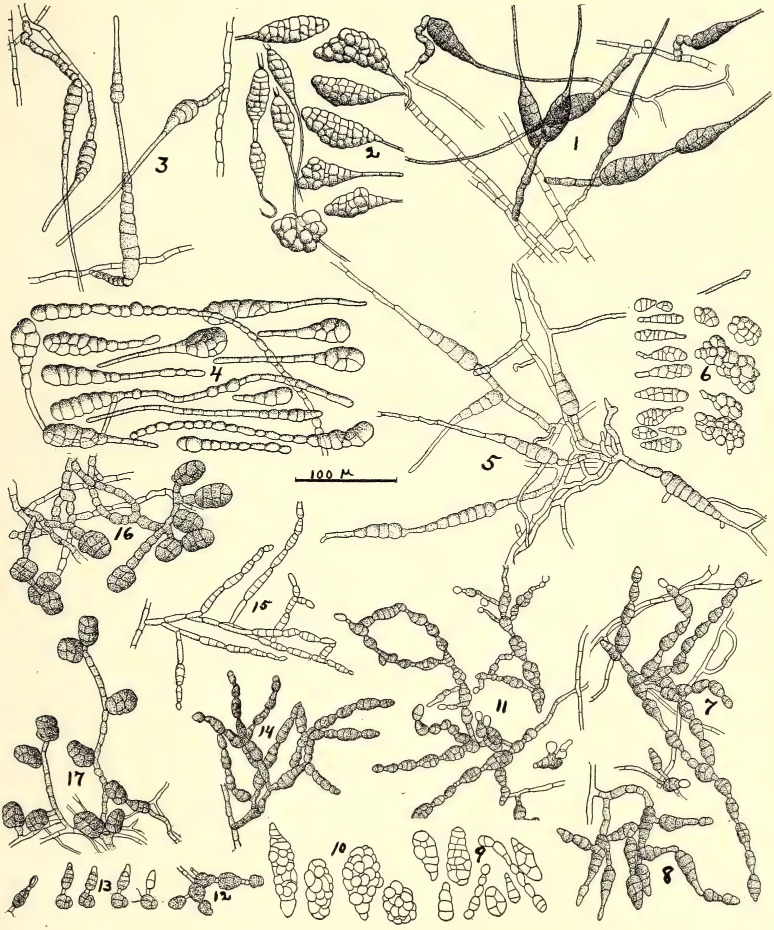
6. Colony of *A. brassicae* var. *microspora* showing reaction to Colony of *B. x.*

7. *A. iridicola* on leached agar.

8. *A. sonchi* conidiophores and spores on *Lactuca canadensia.*

9. *A. sonchi* conidiophores and spores on leached agar.

10. Spores of *A. solani* from Datura; *A*, from Datura leaf spot; *B*, from potato leaf spot; *C*, from tomato leaf spot (artificial inoculations).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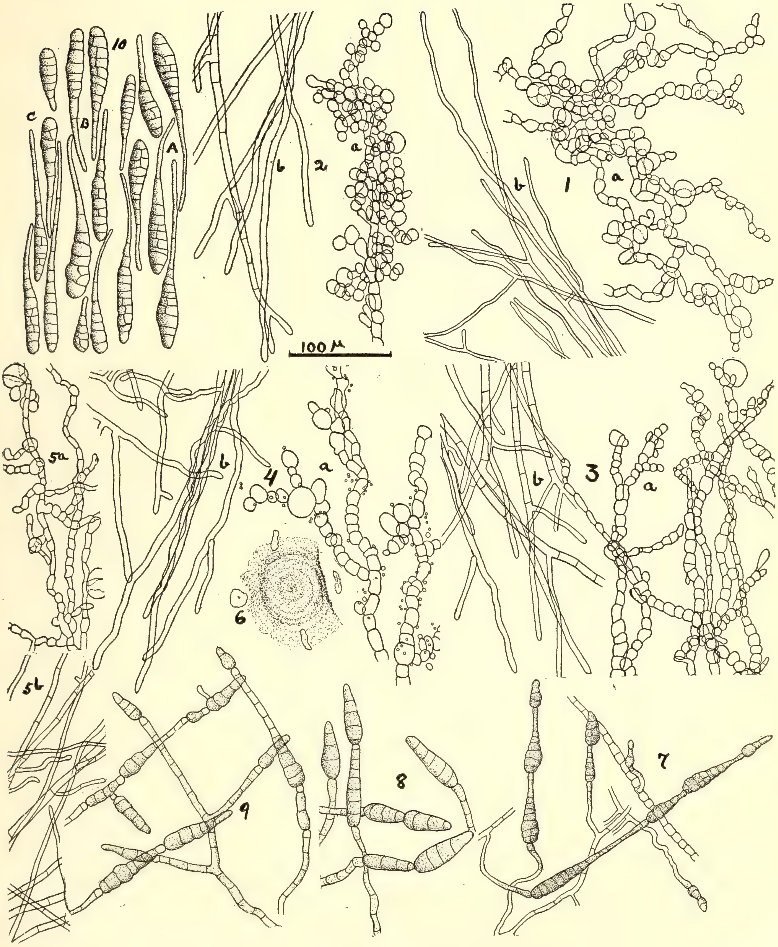
ELLIOTT: ALTERNARIA AND MACROSPORIUM.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ELLIOTT: ALTERNARIA AND MACROSPORIUM.

CROWN-ROT OF FRUIT TREES: HISTOLOGICAL STUDIES

J. G. Grossenbacher

Introduction

The term "crown-rot" is used to designate a bark disease of fruit trees (chiefly apple—*Pyrus malus* and pear—*Pyrus communis*), occurring in most of the tree-growing portions of the United States. The affected bark eventually dies in various sized patches, and the surrounding living tissues produce callus, which thus separates the living tissues from the dead. This disease most characteristically affects the bark of the lower trunk and that of the adjoining portions of the upper roots. The location of affected patches of bark seems to depend very largely upon the interrelation of growth and weather conditions; in some cases the disease involves chiefly the upper roots, while in other cases it occurs most frequently at the base of the trunk. An affected patch of bark that dies to the wood decays more or less rapidly, depending upon its distance from the ground or other sources of moisture. The wood exposed by the decayed bark is usually discolored at its surface but may be alive within and active in the conduction of water. When the crown-rotted patch extends around three fourths or more of the trunk, the downward current of elaborated food in the bark is interfered with to such an extent as to permit much less than the normal amount of radial growth in the roots. The enfeebled roots thus absorb less soil solution, and therefore smaller leaves are formed. The wood under the wound dies in time, and thus the water-conducting tissues are reduced. Such trees usually die in a few years unless radial growth produces much new wood about the wound in the meantime. In some instances the bark dies entirely around the base of the trunk, and in many cases of this sort the width of the dead girdle determines the length of time such a tree will live.

Crown-rot has an important economic bearing upon the fruit industry of this country, owing to the fact that it involves the lives of trees and is therefore much more serious than fruit- and leaf-spotting diseases which after all are essentially matters of a season.

Crown-rot and related bark diseases have been investigated intermittently during many years; in the early days of phytopathology considerable attention was given to these diseases and much valuable information was accumulated. The subsequent enormous development of mycology, in its relation to the diseases of plants in general, has eclipsed and supplanted the interest formerly centered on bark diseases, apparently because the mycological phases afforded more tangible subjects for investigation. Most of the more modern attempts at the study of crown-rot have been of a preliminary nature and have led only to hazy or ill-founded conclusions.

Some of the apparently new ideas that occurred to me during the earlier part of this investigation were published in 1909,[1] while others were stored away, embodied in the form of notes and photographs to be used later. Continued search of the older literature of botany and forestry for observations upon bark diseases, as well as with reference to the question of radial growth,[2] resulted in gradually placing one after another of my supposed new ideas in the category of confirmatory observations and conclusions.

The Literature.—The literature accumulated on crown-rot and related subjects during the past six years has become very voluminous, and to attempt a review seems rather discouraging. Most of the important papers on radial growth, and on certain of the factors determining its distribution, were reviewed some time ago in the last cited paper. Many of the more general papers on this disease and some of those dealing with the cause of the trouble were discussed in my two former papers on crown-rot. There are still too many abstracts of such papers on hand to be fully utilized in this connection, and therefore only a few of the most pertinent ones will be mentioned later in the discussion of my results.

The Causes of Crown-Rot.—The common orchard bark-fungi are evidently the causes of the rotting of the bark in crown-rotted trees, but the cause of the initial injuries that led to the death of this bark has not been experimentally determined, although some work upon this problem has been done on citrus trees in Florida. Field observations in the north, together with a few experiments, have shown that the manner and timeliness of radial growth, pruning, and the occurrence of droughts or low temperatures are closely related to the development of crown-rot there; and similar investigations in Florida are revealing the prevalence of comparable adverse environmental conditions, preceding the occurrence of foot-rot on citrus trees. In the latter case it was also noticed that the leaf-surface area of a tree exposed to sufficient light for active photosynthesis, when compared to the surface area of bark on the branchless portion of the trunk, is often rather small in trees which appear especially subject to this disease; that is, it is suggested that a scarcity of elaborated food coursing downward in the bark of the trunk below the branches may also have a causal relation to the occurrence of crown-rot.

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[1] Crown-rot, Arsenical Poisoning and Winter Injury, N. Y. Agr. Exp. Sta. Tech. Bull. 12: 369. 1909.

[2] The Periodicity and Distribution of Radial Growth in Trees and Their Relation to the Development of "Annual" Rings, Trans. Wisc. Acad. Sci. Arts and Letters 18: 1. 1915.

Methods of Work.—It was found possible to cut usable sections from material of small diameter embedded in celloidin without the previous use of softening agents. The series used in this study was obtained chiefly in the manner described and from material collected and fixed in spring and summer of 1912. The citrus material was collected later in Florida and similarly treated.

Flemming's strong solution and Carnoy's mixture were used almost exclusively for fixation. The former gave more satisfactory material for staining, but the latter was more largely used because of its more rapid penetration. Flemming's triple stain and Heidenhain's iron-alum haematoxylin stain were most frequently used. The triple stain was found to yield much quicker and more satisfactory results when used after a mordant such as iron-alum, but for use in making photographs iron-alum haematoxylin proved more desirable than Flemming's. The same was later found to be the case in sections from foot-rot material of citrus trees.

The Early Stages of the Disease.—The first visible stages of crown-rot consist of discolored and often ruptured tissues variously distributed in streaks and patches in the bark. In cross-section the injured patches are often arranged more or less concentrically about the wood cylinder, although they are usually most severe on one side of the stem. In the mildest forms of the disease the medullary rays of the inner phloem and groups of parenchyma cells about the sclerenchyma strands and inner cortex are affected, although at times only the one or the other of these tissues is involved. In more severe cases much of the phloem and practically all the cambium may be injured.

The severity and course of the disease following these evident beginnings depends mainly upon the relative extent and number of the injured or dead patches, upon the weather of the ensuing growing season, and possibly upon the relative abundance of wound fungi. In many of the milder cases, the injured and collapsed tissues are merely more or less compressed by the subsequent growth of the surrounding live parenchyma cells, and in late summer only the presence of irregular formless dead masses among the living tissues of the bark tells the story of the former trouble. In the more severe cases, however, in which in addition to the medullary rays of the phloem, the inner portions of the cortex and perhaps most of the cork cambium have been much injured, the results are likely to be more serious. In these cases, as in the milder ones, the resumption of growth by the surrounding live parenchyma results in the compression of the dead and dying tissues; but since the dead patches are numerous, relatively large, and close together, the intervening live parenchyma and ray-cells are insufficiently supplied with water and nutrients and therefore cannot survive the drying weather of late spring and early summer. During the latter part of this process a new cork cambium is developed inside the dying cortical parenchyma, resulting afterward in a rough, scaly bark. In cases where the initial injury involves very large patches of outer phloem but leavet the inner phloem and practically all the cambium intact, the resuls is approximately the same, excepting that occasionally small patches of bark die to the wood on account of the occurrence of coincident injured patches in the cambium and inner phloem. It often happens in instances of this kind that the cortex is affected but slightly and that it retains its normal appearance until the internal trouble has become far advanced; then it usually dies rather quickly and dries out. However, none of the types of injury so far described usually result in very serious trouble because at most only small areas of bark are killed to the wood.

When most of the cambium and much of the phloem are initially affected, the injurious results are usually much more evident; but even in such cases the bark may survive if the weather is favorable and if the area affected is not extensive as compared with the total area of the bark of that portion of the stem. In case the injured patches in the cambium and inner phloem are relatively large or fairly close together, or if they form nearly continuous sheaths of affected tissues, the regenerations from the living portions of the bark are hampered by defective water conduction outward from the wood; and unless the weather conditions are most favorable, so that the cells proliferating from the bark are able to re-establish living connections by fusing with the wood cylinder, these proliferating cells die, and the result is the death of the entire bark. Although such patches of dead bark are produced in various ways and result from injuries of varying degree, we term the wounds *crown-rot* if they occur on the bases of trunks or on roots near the ground, and *canker* if they occur on stems and branches above ground.

This histological investigation permits some inferences to be drawn that support most of the important contentions advanced in my former papers; as regards other contentions, however, the evidence is not so convincing. My preparations, as will appear later, indicate that both excessive tensions and certain degrees of immaturity of bark tissues have a causal relation to the development of the initial injuries that give rise to this bark disease. They also substantiate the results obtained by the cultural tests; no fungi are usually in evidence until the middle of May or even later.

The Development of the Disease

The first visible stage of crown-rot, as well as that of some other bark diseases, consists in a discoloration and collapse, or even in a rupture, of groups of tissues mainly of the inner bark. This stage is usually found only in late winter and spring, and is generally not evident to the ordinary observer unless the outer bark is conspicuously cleft. From late spring to mid-summer, however, most of the severe cases attract attention by the oozing of "sap" or gum and by the eventual discoloration of the outer bark. Such affected bark is most commonly found on the trunk near the ground, in crotches, and at the bases of small young branches arising from the large limbs of heavily pruned trees. When at or near the ground, dead bark rots quickly; above ground it usually dries and eventually scales off from the wood.

The initially affected tissues are variously distributed in streaks and patches, which in cross section usually appear in more or less nearly concentric circles about the wood cylinder. In cases of slight injury, the medullary rays of the inner phloem, groups of parenchyma around the sclerenchyma strands or patches of cortical parenchyma are affected. In more severe cases, much of the phloem and all the cambium may be involved or the phloem and cambium injuries may be accompanied by injuries in the cortex. The severity and course of the disease following such initial injuries depend upon the size and number of the affected patches, and upon the location of the most severely affected portions of bark.

The Initial Injuries.—Some of the common types of initial injuries that subsequently give rise to bark diseases are shown on Plate XXI, The group of figures shown on this plate does not, however, include one of the kinds most frequently noticed: these somewhat concentrically arranged injuries often occur with conspicuous radial clefts, as discussed in my former papers, and as indicated in Figs. 43 and 48, Plate VI. In many instances, however, the concentric injuries are not accompanied by radial ruptures, and sometimes radial clefts occur when other types of injuries are so slight as not to hinder subsequent normal bark growth. The sections shown on Plate XXI are all made from apple and pear material collected before growth started in spring (April 17, 1912, at Madison, Wisconsin).

Fig. 1 shows a condition that is frequently found in injured bark. Dead tissues are usually evidenced in these photographs by the occurrence of especially dark streaks or patches, by collapsed cells or by both. Sometimes ruptures are much more prominent than discolorations, as shown in Fig. 3, which shows a very common type of rupture or separation in the inner phloem. In Fig. 1 may be seen a conspicuous combination of the collapse of discolored tissues with ruptures in the inner phloem and cambium. At the left of the section shown in this figure the initial injury is confined chiefly to the cambium; on the right the principal injury occurs in the phloem, only scattered cells in the cambium being affected. A few groups of injured cells may also be seen about the sclerenchyma strands as well as farther out in the cortex. Fig. 2 shows a case from pear tissues in which the initial injury is most pronounced in the inner cortex and outer phloem, with only small groups of affected cells in the outer cortex and inner phloem. Fig. 3 is from apple. It shows a marked injury of medullary rays in the phloem, and a rupture of the phloem.

The other figures on Plate XXI show, on a larger scale, small areas in typically injured bark. In Fig. 4 occurs a mixture of streaks and patches of dead and living tissues present in the outer wood and the inner bark of apple. In the center of this figure a large ray and much of the surrounding tissue is dead and collapsed (appearing black). A portion of the continuation of this ray in the wood is also dead, although its cambial portion is still alive. In the next ray to the right, the outer portion is dead and that in the inner phloem and cambium is alive; but its extension into the wood cylinder is dead and discolored. The phloem and cambium tissues lying between these rays are mostly dead, but scattered living cells occur singly or in small groups. The phloem tissue between the two rays toward the right of the figure are in much better condition, containing only a few scattered groups of dead cells. It is worth noting that toward the right of the figure the main line or *sheath* of injury runs through the outer phloem, while toward the left it affects chiefly the cambium and inner phloem. However, even the most severely injured sheath has living cells interspersed among the dead and collapsed ones. In Fig. 5 such is not the case; here the sheath of injured tissue involves the cambium and inner phloem on the left; at about the middle of the figure it bends suddenly and proceeds through the phloem, leaving the cambium alive except for occasional groups of injured cells. In this instance the sheath of most severe injury contains few living cells, thus practically eliminating all living connection between the wood, or its living fringe of cambium, and the bark proper outside the injured sheath. Fig. 6 shows a comparable condition excepting that more dead cells are scattered among the living tissues. In this instance the broad ray near the center is dead in both bark and wood, and on the left the entire cambium and the inner phloem, with the exception of a few cells, are collapsed. The outer cortex shows many dead cells. Fig. 7 gives a better idea than the others of the injury occurring in the inner bark: here the cambium and inner phloem as well as the rays and much other phloem tissue are dead and more or less collapsed. In the cambial region near the left, however, is a group of two or three living cells with irregular outlines. These cells, as well as those of certain groups in Figs. 4 and 5, are apparently enlarged, although the apple trees from which this material was cut seemed perfectly dormant at the time. It appears likely that this represents the beginnings of spring growth and regeneration, brought on early as a response to the wound stimulus. In Fig. 8 occur two lines or sheaths of severe injury, one of which involves the cambium and the other the outer phloem. Neither of these zones is made up wholly of dead cells, so that the sheath of living phloem between them is not entirely without living connection with the wood and the outer bark. Fig. 9 shows a condition much like that in Fig. 7, excepting that larger masses of inner phloem are alive, although not evidently affording living links between the bark and the wood. In both Figs. 7 and 9 the sheath of dead tissue seems complete, thus isolating the bark from the wood cylinder at these places.

The initial injuries presented in Plate XXI are not shown because they represent the most severe cases, but because the location of the injuries is typical and yet they are not severe enough to prevent proper handling of the sections. As noted above, material for sectioning had to be taken from portions of trees where the areas of individual injuries or dead patches were relatively small in order to prevent the shattering of the blocks before they were imbedded. When small blocks were cut from the very edge of one of the more extensive injured areas, they frequently remained intact through the imbedding and sectioning processes; if, however, the entire block was within such an area, its different portions usually fell apart, separating along the planes of severest injury. This falling apart of the blocks was less troublesome in the material collected May 1 than in that collected April 17. The blocks cut on May 29 from the more severely affected and larger areas were extremely fragile, while those from regions of less injury were more stable than specimens of the same degree of injury collected April 17.

Some Changes Due to Growth and Regeneration.—The figures of Plate XXII are made from photographs taken of sections of apple collected May 1, 1912. These show some interesting phenomena of growth and regeneration, and among other things suggest how and why it is that so few bark injuries give rise to dead patches of bark.

In Figs. 10, 11 and 12 are shown cases in which the initial injury involved all or nearly all of the cambium and a portion of the inner phloem, with dead streaks of less extent scattered in other parts of the phloem. In all three of these cases subsequent regeneration growth from the living portions of the phloem resulted in establishing a more or less definite living connection through the zone initially involved. As a result of this growth, the material in the scattered dead streaks in other portions of the phloem has become compressed into ragged plates with their edges directed toward the wood. In other parts of the bark dead groups of cells are similarly compressed by the more or less bladdery growths from the surrounding tissues. In Figs. 10 and 11, comparatively few of the proliferating bark-cells appear to have established a close contact with the old wood; but in Fig. 12, representing a section in which the dead zone had not been so wide and still contained a few living cells, the new growth is much more firmly attached to the old wood. Fig. 13 again is more like Figs. 10 and 11, although its dead or injured zones are less extensive; in the section here shown, too, some living cells capable of further growth appear, which are attached to the old wood and have grown outward to meet the growth from the bark. In the tissues shown in Figs. 10, 11 and 12, the initial injuries had been most severe in the cambial and inner phloem regions and are of the type shown at the left in Fig. 1, Plate XXI; while Fig. 13 shows a regenerated condition of an initial injury more like that indicated in Figs. 7 and 9, Plate XXI, where some living bark cells or cell-groups remain attached to the wood. In Fig. 14 is shown an effort toward recovery that is rather remarkable and far advanced for May 1. This represents a reaction to wounds of the type shown near the right in Figs. 1, 3, 4, 5 and 6, Plate XXI. In addition to the general compression of the dead tissues by the growth of the living cells around them, many proliferating cells have pushed in among the portions of the dead sheath, thereby facilitating the re-establishment of living connections between the outer bark and the living inner phloem that remains attached to the wood-cylinder. Considerable injury also occurred in the cambium, although small portions of the latter appear to have survived. A new cambium, however, is seen to be forming outside among the irregular cells arising from the wound growth. The figure does not show it as clearly as the microscope; it is beginning to take form in the line *cc*. Fig. 15 represents a similar instance, except that the initial injury was more extensive and that larger groups of dead cells resulted. A new cambium is forming at *cc*, though it is incomplete and still has compressed fragments of dead tissue in its course. Fig. 16 seems to be a later stage of a case something like the left-hand portion of Fig. 8, where the cambium was only slightly injured and the outer phloem rather severely, though in more or less isolated streaks and patches. Some of the rays are dead, although a few are practically normal, like that near the right of Fig. 16. The new cambium is quite distinctly indicated by the dense band *cc*. In Fig. 17, comparable but severer initial injuries obtained. The re-established living connections between the growing phloem and the wood are few and scattered, and the injury in the outer phloem forms a nearly complete sheath, thus isolating the cortex considerably from the phloem. A scattering of living cells occurs, however, in this outer-phloem zone to afford water and nutrient transfer between the outer and inner barks sufficient to permit the outer bark to endure at least for a time.

The figures of Plate XXII likewise give only a few of the great variety of the injuries that were mild enough to permit more or less regenerative growth during the spring, although some of the figures plainly indicate that only a most favorable summer would enable the affected bark to survive.

Some Results Found at the End of May.—Plates XXIII and XXIV give an idea of the great variety of results following some of the initial injuries shown on Plate XXI. The low-power views collected on Plate XXIII make it evident that regeneration and growth of the living cells are not all that is required to sustain the affected bark and to keep it from dying in early summer. Figs. 18 to 22 inclusive show some of the milder forms, while Figs. 23 to 26 indicate various stages and degrees of injury resulting in the death of patches of bark.

Fig. 18 shows an advanced stage of an initial injury of the type shown in Figs. 7 and 9, and, in later condition, in Figs. 10 and 11. The new cambium sheath arose much after the manner shown in Fig. 17. The compressed fragments of dead tissues, present at the time spring growth started, are noticeable in the new wood (*nw*) as well as in the old phloem (*op*). The initial injury was so severe that the old wood and bark-rays were not continued by the new growth; new rays are just becoming differentiated on both sides of the new cambium (*nc*). In this case the outer bark seems to have established sufficient living connection with the regenerating inner bark to continue its normal functioning, but the connection between the old (*ow*) and the new wood (*nw*) seems to be insufficient in places, for the new cambium (*nc*) has also developed in the inner phloem. In this case the initial injuries in the outer phloem and cambium were so extensive as greatly to delay the development of the new cambium (*nc*), as seen near the middle of the figure. No definite new wood cells have yet been formed at this point, although on both the right and left sides a considerable layer of new wood has resulted and the new cambium appears practically normal. Fig. 20 shows some interesting irregularities in the distribution and configuration of the initial injuries. They had evidently been of the type shown in Figs. 1, 5 and 9, and the new cambium apparently started to form in the inner phloem just outside the dead cambium as shown by the light line near the wood, at the lower left. But because of marked irregularities in the distribution of the sheath of severest injury, the first new cambial initial was supplanted by one farther removed from the wood and overtopping the irregularities, as shown at *nc*. Toward the left of this figure the injury had been so severe as wholly to inhibit the development of a new cambium, although the cell proliferation had occurred that ordinarily precedes the production of a new cambium. Some of the small groups of living cells in that region had become light brown in color and were evidently dying. Toward the left end of the new cambium only scattered groups of new wood cells had developed. The portion of old inner phloem, separated from the rest by the mantle of injured tissue (near the center), had grown somewhat, but no definite cambium had been evolved. In the section shown in Fig. 21 similar isolated strips had developed into wood cells, even though the new cambium, as in Fig. 20, developed farther out in the phloem and produced a rather broad layer of new wood outside the zone of greatest injury. This type of figure seems to have been developed as the result of initial injuries like those shown in Figs. 5, 7 and 9. Much dead tissue was compressed into irregular masses in the outer phloem and cortex. The new cambium (*nc*) has a brownish tinge and seems to be much collapsed. It should be noted in this case that the phloem left attached to the old wood was transformed into wood without leaving a cambium. Fig. 22 is interesting chiefly on account of the fact that injuries in the outer cortex resulted in the development of a new phellogen layer or cork cambium within (*ph*). Fig. 2, Plate XXI, shows an injury occurring mainly in the inner cortex, that is often similarly cut off by a phellogen developing in the outer phloem. Fig. 23 is somewhat comparable to the left portion of Fig. 20, in that no new cambium has developed, although considerable regeneration growth has occurred. The cortex is practically uninjured and therefore appears normal from the outside, but both the outer and inner phloem are severely affected and the cambium is entirely dead, except in isolated streaks like that shown near the left. But even in this severely injured phloem occur groups of living cells, though they are more or less completely isolated by dead tissues. Many enormous bladdery outgrowths from the living cells are forced into the dead masses. In some places the living and in others the dead tissues predominate in the phloem. It may be noticed that on the left the bark is thicker than it is on the right. This results from the presence of larger groups of living cells in this portion of the phloem; indeed, it appears that groups of wood cells (appearing in the photograph as rather dim whitish patches) have in some way arisen in this region. The process had advanced further in the specimen shown in Fig. 24. Here some of the bark had died to the wood, and, because of the presence of much dead tissue in the inner phloem and the old cambium, the callus is a rather sickly affair. It includes the repaired phloem considerably speckled with masses of dead tissue, and the badly injured cortex. In the lower right corner occurs a strip (white) where the phloem is being transformed into wood cells, yet no definite cambium is in evidence. Although not shown in this figure, the slide from which this photograph was made shows abundant hyaline fungus mycelium in the dead bark, even in the dead cambium and the old inner phloem between the callus and the old wood. Fig. 25 shows a similar case in which the most severely affected area was very narrow, thus permitting its use in this study without its falling apart. In this instance the callus is much better developed, having a definite cambium and a layer of new wood. The old inner phloem and the old cambium were also dead for some distance back of the nose of the callus. Some fungus mycelium was present in the dead bark. Fig. 26 is made from the margin of a larger area that had sunken in, like that shown in Fig. 41 of Plate XXV. The marginal callus was much like that of Fig. 25, and the presence of fungus is indicated by the pycnidia (of *Sphaeropsis?*) showing under the periderm toward the left.

On Plate XXIV are brought together some higher-power views giving greater detail, though in some instances cell outlines are necessarily more hazy. Figs. 27 and 28 show clearly the remains of the dead cambium and inner phloem; they also prove that even as late as the end of May substitution growth is in progress along the inner side of the repaired bark and has established better connection with the old wood. This latter fact is indicated by the presence of excessively large round cells, that appear to be filling the gaps left by the shrunken dead tissues. Fig. 30 shows the development of a new cambium (*nc*) between the dead inner phloem and sheets of dead tissue in the outer phloem. In Fig. 31, groups of dead cells appear in the former position of the inner phloem, around and among which some of the new wood (*nw*) cells are becoming discolored. This intermingling of living and dead groups of cells is most common in the phloem. The position of the new cambium (*nc*) is occupied chiefly by structureless masses having a yellowish brown color. The cortex and outer phloem had only scattered groups of dead cells. No fungous hyphae could be detected in this specimen. Fig. 32 shows tissues which had sustained more severe initial injuries but which, because of the small area of the most severely affected part, endured quite well, while in the case of milder injury but of larger area shown in Fig. 31 the tissues seemed to be dying. In the tissues shown in Fig. 32, the outer phloem also is largely killed but the cortex is in fair condition. A new cambium (*nc*) is forming near the old wood. At this place no living connection appears between the bark and the wood, but because of the smallness of the region so severely affected, the necessary water and nutrients seem to reach it from the sides of the injured patch where such connections do occur. Fig. 33 shows a case in which much of the inner phloem had been killed and in which very active filling-growth is occurring. A faint indication of the formation of a new cambium can be seen here and there; a spot of this kind appears near the center of the figure. The photograph from which Fig. 34 was made shows a very large group of dead phloem tissue which has been only partly permeated by proliferating cells arising from living adjoining cells. In some cases the initially killed strips of tissue form an anastomosing network lengthwise through the bark; in extreme instances only anastomosing streaks of inner bark may be alive while the greater mass of the bark is dead. A cross section of such a living streak in great masses of dead tissue may be seen in Fig. 35. In this instance large groups of dead cells also occurred in the inner cortex, although when the specimen was cut (May 29) its external appearance was practically normal.

From another type of initial injury enlargements as well as wood-exposures occur on trunks of trees. Such cases of excessive enlargements on trunks may sometimes develop from initial injuries of the type shown in Fig. 3, Plate XXI, in which a definite separation or a concentric cleft has resulted, and where at the same time the repair growth and connection with the wood are such as to prevent the dying of the loosened outer bark. When radial clefts run through such areas and the bark is otherwise sufficiently intact to withstand the drying action of air, the repair growths may turn the edges of the loose bark outward, while if no radial cleft occurs the enlargement may look like that on a maple trunk shown in Fig. 40, Plate XXV. The resulting repair growths are not always uniform even when they follow the kind of initial injury that separates the bark from the wood. In some cases the loosened bark has but few injuries (Fig. 3, Plate XXI); in other cases, or perhaps even in other portions of the same affected area, the outer phloem as well as the cortex may have so many groups of dead cells scattered among the living parts that the entire bark dies. That has been the case in the lower portion of the stem part shown in Fig. 40. A section through the upper part of this maple trunk reveals a condition like that shown in Fig. 37. Here the discolored line *oc* represents the position of the cambium when the injury occurred. The initial injury not only resulted in a line of separation in the inner phloem like that shown in Fig. 3, but involved cell-groups in the cambium proper as well as in the middle and outer bark. As in the case shown in Fig. 3, however, the inner phloem had sufficient living connection with the wood to permit the development of a cambium that persisted, excepting in the bare region shown toward the lower end of Fig. 40, where it died along with the loosened bark outside. A cambium also developed in the inner part of the loosened bark shown in Fig. 3, running through the outer phloem. After the production of a sheath of new cells in this new growing zone, the middle ones became wood and those along *both outer* sides continued as cambial zones. In that way *one* growing zone was converted into *two*, which separated more and more as the older cells toward the middle were converted into wood. The sheath of new wood (*nw*) just within the old outer bark (*ob*, Fig. 37) arose in that manner, and has a cambium on each side. The low-power views shown in Fig. 36 (*a–e*) are photographs of sections cut from blocks obtained from the specimen shown in Fig. 37. Fig. 36*a* shows the old outer bark (*ob*) of Fig. 37, with only a small portion of the new cambium (*nc*) included. The new bark shows compressed inclusions of dead tissues resulting from the initial injury. Figs. 36*b* and 36*d* are so tightly pressed against each other in Fig. 37 that the two barks seem to be one. Fig. 36*e* is taken from the line *oc* in Fig. 37 and shows more clearly the similarity of this line to the figures obtained from apple, such, for example, as Fig. 18. Fig. 39 is a more highly magnified view of a portion of Fig. 36*e*. It shows the presence of dead groups of old cambium cells adhering to the old wood (*ow*).

It is evident that in most places along the line *oc* of Fig. 37 the cambium, giving rise to the new wood (*nw*) outside it, also arose much like that shown in Figs. 14, 15, and 17, from cell divisions occurring in the adhering layer of phloem (Fig. 3). Trécul[4] seems to have obtained very similar results by pulling loose and then tying back a piece of bark.

Fig. 39 shows another fact of special interest, for it makes it apparent that much of the new wood formed about the original line of injury subsequently dies and becomes discolored. Even in portions of the line *cc* of Fig. 37, where the new wood is practically continuous with the old and where the rays are not even interrupted, there has been more or less of this discoloration and dying of the new wood, as shown in Fig. 42. Here the initial injury seems to have involved chiefly the outer end of the wood rays and scattered, but small, groups of cambium cells. Nevertheless a narrow, ragged-edged zone of new wood died and became discolored, as Fig. 42 shows.

The Development of Crown-Rot from the Initial Injuries.—As suggested above, bark-injuries may or may not be accompanied by evident radial clefts, and when they are not evident externally they are yet often present, as may be gathered from Fig. 36*a*. The old bark (*ob*) is shown to be run through by numerous small, radial rifts that represent incomplete ruptures which were afterwards filled by proliferations from adjoining cells. Fig. 38 shows a case of this kind, also in maple, where apparently the bark was only separated from the wood, and yet where so many of these tiny radial rifts occurred that the bark involved is evidently dying. In this case the whole of the cambium as well as much of the inner phloem died as a result of the initial injury. The rest of the bark was still alive when cut on May 28. Although no definite new cambium had yet developed in this loosened portion of bark, the spring growth of wood is seen to be considerable on both sides of the wound. Callus-roll formation has made an evident beginning around the injury, even though the bark involved is not dead.

On the other hand, Figs. 43 and 45, Plate XXVI, show apple trees in which internal bark-injuries, resulting in a separation of the bark from the wood, were accompanied by evident radial clefts. The former is shown before and the latter after the loose bark was removed. Fig. 44 shows the same tree as that in Fig. 43 with the loose bark removed, making it apparent that the tree was half girdled, though only a fairly narrow band (not exceeding 17 cm. in vertical width) was involved. The loosened bark shown as removed in Fig. 45 had several short radial clefts, though otherwise the bark appeared normal while it was still on the tree. When removed, the inner surface of the bark and the wood thus exposed had a rusty brownish color. On cutting with a knife it was found to contain numerous closely scattered, small dead spots, which in some places had coalesced to form ragged, dead patches as much as one or two centimeters in diameter. These patches often involved all but the outermost layers of the cortex and sometimes showed through the periderm or scaly outer bark in the form of dead spots from one to five millimeters in diameter. In places, however, this loose bark was found to have developed a new cambium in addition to bladdery outgrowths from the inner phloem, thus tending to re-establish connection with the wood cylinder. Similar, though scattered, outgrowths had also developed from outside the wood cylinder, but the actual connection established was evidently slight; for on June 24, when it was removed, considerable areas of these proliferations had died and turned a rusty brown. In fact, disorganization seemed to have set in over a large part of the inner surface of this bark.

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[4] Trécul, A., Production du bois par l'écorce des arbres dicotylédonés, Ann. Sci. Nat. Bot. III **19**: 257. 1855.

When, on May 7, the loosened bark was removed from the tree shown in Figs. 43 and 44, only a slight discoloration was noticed on the contact surfaces. The loosened bark appeared perfectly normal on the outside, with the exception of the presence of a wide radial cleft. Numerous scattered groups of dead tissue were found in the older phloem and inner cortex. Proliferation growth had been abundant, and in a few places it appeared that cambium was in process of formation.

In cases of injury in which the affected bark does not die and where but *one* substitute cambium develops, only the discolored line in the wood afterward remains as a permanent record. This line is marked *oc* in Figs. 18 and 19, Plate XXIII, and 37, Plate XXV. Fig. 49, Plate XXVII, is an especially clear illustration. It represents a cross-section of the base of a large apple-tree trunk from an orchard in which the initial injuries, leading to the development of crown-rot, had occurred on many trees the same number of years back, as is indicated by the radial-growth zones outside the conspicuous line of discoloration in the wood. It should be noted also that at least one radial cleft occurred in the bark of the tree represented in Fig. 49: the fusion is still incomplete. However, in the tree, a section of which is shown in Fig. 50, a portion of the affected bark died and the entire wood cylinder, up to the line represented by *oc* in Fig. 18 (the outer boundary of the wood at the time of injury), has completely decayed. In a few points, just as in Figs. 3, 39 and 42, some of the wood produced afterward also died and decayed. This shows that decay depends as much upon the death of the wood as upon the presence of wood-rotting fungi. Bark and wood having groups or streaks of dead tissue naturally contain relatively large quantities of air, and sapwood dying from such bark injuries is full of both air and stored food. The high air content of such wood led Münch[5] to conclude that the presence of the excessive air is the factor that permits wood-rotting fungi to vegetate actively in such tree trunks. Based on these conclusions of Münch, Appel[6] has been led far afield in his discussion of the factors governing the activity of wood-rotting fungi. The fact that such wood is killed while it is young and full of stored food makes it evident that it differs materially from ordinary heartwood that has become depleted of most of its stored food (including the layers of hemicellulose usually present on the inside of its cell-walls) before it became lifeless. It seems more likely that wood-rotting fungi thrive uncommonly well in such wood because it contains large quantities of stored food and masses of more or less disorganized and therefore non-resistant protoplasm, rather than because of the great abundance of air present.

The small apple tree shown in Fig. 46 and the large ones of Figs. 47 and 51 are examples in which the most severely affected bark died. In those shown in Figs. 26 and 51 a complete girdle is involved, while in that of Fig. 47 only about three fourths of the bark succumbed.

Comparison of Effects on Large and Small Trees.—The initial injuries, from which crown-rot and some other bark diseases arise, are the same on large and small trees; the differences usually noticed afterward result from subsequent changes owing to differences in the thickness of the bark and in the diameter of the stems or branches involved. It is also shown that if the area of the bark most severely affected is large compared with the circumference of the stem involved, the result is more serious than when the injured patch is comparatively small. This holds for both large and small, as well as for young and old stems. If the section from which Fig. 26 is made were photographed whole and magnified, a picture much like Fig. 50 would result, although the wood cylinder within the injured zone was not decayed or even entirely dead when cut on May 29. The new growth of wood shown in Fig. 26 as well as that in Fig. 25 consisted of only a very thin layer, while the wood shown in Figs. 49 and 50 represented several years' growth. Fundamentally, however, these sections are not only comparable but very similar.

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[5] Münch, E., Untersuchungen über Immunität und Krankheitsempfänglichkeit der Holzpflanzen, Naturw. Zeit. Forst. Landw. **7**: 54; 87; 129. 1909.

[6] Appel, O., The Relations between Scientific Botany and Phytopathology, Ann. Mo. Bot. Gard. **2**: 275. 1915.

The Cause of the Initial Injuries

The work so far has clearly shown that the initial injuries in the bark of trees that result in crown-rot arise during the dormant season, but their cause has not been definitely established. The years of observation and a few experiments together with the histological study here reported, indicate the most probable factors, and thus pave the way for an experimental study of the problem. In general terms it may be said that these initial injuries are due to a lack of adjustment between radial or bark-growth and the environment.

Some Facts about Bark-Growth as Related to the Development of These Injuries.—In the study of forest trees it has been shown that growth and development proceed in a wave-like manner. The various functions, the size of cells, and the amount of annual growth increase to maxima in certain stages of a tree's life, and decrease to minima again at other stages. These periods or cycles are repeated at intervals more or less characteristic of a species. Kapteyn[7] calls attention to growth cycles that may be traced in the wood and extend over periods of 12.4 years, apparently independent of the species. Bailey and Shepard[8] found that the length of coniferous tracheids varies in more or less definite cycles usually ranging from 35 to 80 years, and apparently differing in different species.

It is a well-known fact, for instance, that at a certain age of a woody plant the development of primary bark is supplanted by the formation of secondary bark, apparently because of the inability of the primary tissues to continue their adjustment to the increase of the stele. This transition stage in trees usually occurs at an age characteristic for the species. Afterward the bark continues to increase in thickness, and to be distended, growing somewhat radially for a certain number of years; then a new phellogen is again formed inside that portion of the bark that is no longer able to undergo sufficiently rapid tangential growth, and a fresh portion of lifeless bark results which is more or less continuous around the trunk—the results in this respect also varying with the species.

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[7] Kapteyn, J. C., Tree-Growth and Meteorological Factors, Réc. Trav. Bot. Néerland. 11: 70. 1914.

[8] Bailey, I. W., and Shepard, H. B., Sanio's Laws for the Variation in Size of Coniferous Tracheids, Bot. Gaz. **60**: 66. 1915.

It appears that there is a close relation between the growth cycles mentioned above and the periodicity that may usually be noticed in regard to bark cycles. The growth and cell-size minima seem usually to fall in the season just preceding a new period of bark-roughening, while the maxima are usually reached during the second third of the time elapsing between the occurrence of two minima. The environmental variations, and, in case of cultivated trees, the culture and tilth given, have a marked influence upon the prominence of this periodicity.

In a smooth-barked stem that portion of the bark outside the oldest circle of sclerenchyma (the cortex) often undergoes much, although limited, growth. Sections of apple and pear stems from material fixed at different times of the year indicate that during seasons of much or of late radial growth cortical growth sometimes continues very late and is not completed by the time the period of dormancy arrives. The increase in diameter of wood necessitates and *is followed by* an increase in the area of the bark. When an adverse change in the weather conditions interferes before this cortical growth is completed, the dormant period must be passed with the outer bark in this unfinished condition. In such a case the bark is often under considerably higher transverse tension than it is in cases in which its cortical growth has been finished. In instances also in which bark growth has been very slight during some years, the cell walls of the cortical tissues and those in the outer phloem are thickened to such an extent that a rather rapid resumption of radial growth is not immediately followed by cortical growth, and therefore high bark tension ensues. If such hardened outer bark is eventually forced into growth late in the season, some of the cells necessarily pass through the dormant season in immature condition, and thus are likely to become injured. Nördlinger[9] found by peeling tests that cambial activity precedes cortical growth and may continue after cortical growth ceases. In some cases, however, cortical growth continued later than cambial activity.

R. Hartig[10] describes several cases in which the bark of a very high percentage of forest trees was burst and injured at certain places two years after those forests had been thoroughly thinned. After thinning, the trees grew as much in one year as they had grown before in many years. His conclusion, that the bark burst in early summer owing to the rapid radial growth of the wood, can fortunately be more carefully examined because he gives a photographic record of cross-sections. These figures prove beyond question that the bark was split and separated from the wood during the *dormant season preceding* the growing season in which he assumes the splitting to have occurred. From Fig. 52, Plate XXVII, which is a reproduction of one of Hartig's figures, it is apparent that the bark injury occurred *between* the growing seasons and not while growth was going on because the lines of injury and separation coincide with the line separating the wood of two growing seasons. Another case which Hartig gives in some detail, in which a high percentage of the trees in a thinned forest sustained bark injuries just above or at the ground line a few years after thinning, is also of decided interest. In this instance he concludes that the rank growth of herbaceous plants developing about the tree trunks after thinning prevented proper aeration, excluded light, and thereby injured the bark. But in this case as in the former, cross-sections show that the injury occurred during the dormant season, when aeration was probably good. The chief difference between these two cases lies in the fact that in the former instance the tension reached a high enough point to rupture the bark as well as to loosen it, while in the latter the tension was less. It seems possible that in one instance the bark was more resistant to radial rupture than in the other, though it is likely that some additional factors are involved in the occurrence of radial clefts.

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[9] Nördlinger, H., Wann beginnt Bast, wann Lederschicht der Rinde sich zu lozen? Centralbl. Gesamt. Forstwes. Wien. 5: 128. 1879.

[10] Hartig, R., Zersprengen der Eichenrinde nach plötzlicher Zuwachssteigerung, Untersuch. Forstbot. Inst. München 1: 145. 1880.

——, Das Zersprengen der Hainbuchenrinde nach plötzlicher Zuwachssteigerung, Untersuch. Forstbot. Inst. München 3: 141. 1883.

Environmental Factors Having a Causal Relation to the Injuries.—My work in northern orchards showed that bark injuries which are caused by the combination of immaturity of tissues and the occurrence of low temperature may give rise to crown-rot. In Florida the occurrence of a temperature only about two degrees or less below the freezing point of water, when certain bark tissues of citrus trees are immature, may result in similar injuries and give rise to equally destructive diseases. Severe droughts often cause similar injuries. When the bark of citrus trees is dormant, it will endure temperatures even below – 10° C. and severe droughts without serious injury.

It is still uncertain whether this bark injury is due chiefly to simple physical causes, such as contraction or to chemical and physiological changes induced in the protoplasm by low temperatures and drought, or to both sets of factors acting together. As a matter of fact, changes of both kinds take place in plants subjected to low temperature and untimely droughts, and we have fairly tangible evidence that both may be injurious.

The photographs submitted with this paper give ample evidence that high tensions, and even ruptures, accompany some at least of the more severe bark injuries. Trunk measurements previously published also show the occurrence of high tensions.[11] In some cases, however, no actual ruptures appear to result, and yet tissues become discolored before the commencement of the next vegetative period. Injurious low-temperature tensions of less degree than those required to rupture the bark are evidently of frequent occurrence, and these are apparently responsible for much of the bark injury afterwards resulting in disease. An extreme form of this effect is shown in Fig. 37*a*. Some of the milder tensions are also shown in Figs. 3, 4 and 5 of Brown's[12] recent paper. Sorauer[13] has given much attention to this type of injury and even applies his low-temperature tension hypothesis to cold injury of herbaceous plants.

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[11] Crown-Rot of Fruit Trees: Field Studies, N. Y. Agr. Exp. Sta. Tech. Bull. **23**: 36. 1912.

[13] Sorauer, P., Experimentelle Studien über die mechanischen Wirkungen des Frostes bei Obst- und Waldbaümen, Landw. Jahrb. **35**: 469. 1906.

——, Weswegen erkranken Schattenmorrellen besonders leicht durch Monilia? Zeit. Pflanzenkr. **22**: 285. 1912.

——, Einige Experimente zum Studium der Frostwirkungen auf die Obstbäume, Die Naturw. **1**: 1055; 1094. 1913.

[12] Brown, H. P., Growth Studies in Forest Trees. 2. Pinus Strobus, Bot. Gaz, **59**: 197. 1915.

——, Altes und Neues über die mechanischen Frostbeschädigungen, Zeit. Pflanzenkr. **24**: 65. 1914.

Some interesting advances have been made in recent years in the study of the chemical and physiological side of this question, but unfortunately the investigators interested in this phase of the subject have thus far given no attention to the more simple physical concomitants presented by Sorauer in the papers just referred to. It is in fact usually assumed that the earlier works had decided this matter. Nägeli,[14] for example, made some studies of this type and concluded that since walls of Spirogyra cells killed by low temperature are not ruptured, death must be due to changes induced in the protoplasm. Kunisch[15] maintained that low temperatures induce harmful irreversible changes in certain components of the protoplasm that result in the death of tissues; that in some plants such changes may even occur above the freezing point,[16] although in others a temperature much below freezing is necessary to cause injurious effects. Fischer,[17] after very fully discussing the literature and giving the results of his own extensive experimental study of the problem, concluded that the low-temperature death-point of plants usually does not vary more than two, though it may vary as much as ten, degrees. On the other hand, Winkler[18] found that the condition of the protoplasm at the time of the occurrence of the low temperature has much to do with the degree of resistance or injury.

Some have held that low-temperature injury results from ice-formation; others believe that it is the withdrawal of water during freezing that causes the injury.[19] Apelt and others[20] have brought

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——, Über Frostschorf an Apfel- und Birnenstämmen, Zeit. Pflanzenkr. **1**: 137. 1891.

[14] Nägeli, C., Ueber die Wirkung des Frostes auf die Pflanzenzellen, Sitzungsb. Akad. Wiss. München **1**: 264. 1861.

[15] Kunisch, E. H., Ueber die tötliche Einwirkung niederer Temperaturen auf die Pflanzen, Inaug. Dissert. Breslau. 1880.

[16] Molisch, H., Untersuchungen über das Erfrieren der Pflanzen. Jena. 1897.

——, Das Erfrieren von Pflanzen bei Temperaturen über dem Eispunkt, Sitzungsb. Akad. Wiss. Math. Naturw. (Wien) **105**: 82. 1896.

[17] Fischer, H. W., Gefrieren und Erfrieren, eine physico-chemische Studie, Beitr. Biol. Pflanz. **10**: 133. 1911.

[18] Winkler, A., Über den Einfluss der Aussenbedingungen auf die Kälteresistenz ausdauernder Gewächse, Jahrb. Wiss. Bot. **52**: 467. 1913.

[19] Müller-Thurgau, H., Ueber das Gefrieren und Erfrieren der Pflanzen, Landw. Jahrb. **9**: 133. 1880.

out some very pertinent reasons for their contention that low-temperature injury is not due to the withdrawal of water.

Even in much of the older literature on this subject, one finds interesting comments on the apparent changes that living substances and stored foods of plants undergo on the gradual approach of cold weather. It was noted, too, that plants thus modified are more resistant to low temperature. The more modern work on this phase of the subject, by Gorke[21] and Lidforss,[22] as well as the work by Bartelzsko,[23] formed a basis for the most interesting researches of Maximow.[24] The latter author showed that it is not the molecular concentration of the cell sap that is most significant, but that the physical nature of the solutes determines the degree of resistance afforded a plant. It was found that the introduction into plant

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Greeley, A. W., On the Analogy between the Effects of Loss of Water and Lowering of Temperature, Amer. Journ. Physiol. **6**: 122. 1902.

Matruchot, L., et Molliard, M., Modifications produites par le gel dans la structure des cellules végétales, Rev. Gen. Bot. **14**: 463; 522. 1902.

Bruijning, F. E., Zur Kenntniss der Ursache des Frostschadens, Forsch. Gebiete Agr. Phys. 19: 485. 1896.

Chandler, W. H., The Killing of Plant Tissue by Low Temperature, Mo. Agr. Exp. Sta. Research Bull. **8**: 143. 1913.

[20] Apelt, A., Neue Untersuchungen über den Kältetod der Kartoffel, Beitr. Biol. Pflanz. **9**: 215. 1909.

Rein, R., Untersuchungen über den Kältetod der Pflanzen, Inaug. Dissert. Halle. 1908.

Voigtländer, Hans, Unterkühlung und Kältetod der Pflanzen, Beitr. Biol. Pflanz. **9**: 359. 1909.

Mez, Carl, Neue Untersuchungen über das Erfrieren eisbeständiger, Pflanzen, Flora **94**: 8. 1905.

[21] Gorke, H., Über chemische Vorgänge beim Erfrieren der Pflanzen, Landw. Vers. Stat. **65**: 149. 1907.

[22] Lidforss, B., Die wintergrüne Flora, eine biologische Untersuchung, Lunds Universitets Årsskrift, N. F. 2^2, nr. 13. 1907.

[23] Bartelzsko, H., Untersuchungen über das Erfrieren von Schimmelpilzen, Jahrb. Wiss. Bot. **47**: 57. 1910.

[24] Maximow, N. A., Chemische Schutzmittel der Pflanzen gegen Erfrieren, I. Bericht. Deutsch. Bot. Ges. **30**: 52. 1912.

——, Chemische Schutzmittel der Pflanzen gegen Erfrieren, II. Die Schutz. wirkung von Salzlösungen, Bericht. Deutsch. Bot. Ges. **30**: 293. 1912.

——, Chemische Schutzmittel der Pflanzen gegen Erfrieren, III. Über die Natur der Schutzwirkung, Bericht. Deutsch. Bot. Ges. **30**: 504. 1912.

——, Experimentelle und kritische Untersuchungen über das Gefrieren und Erfrieren der Pflanzen, Jahrb. Wiss. Bot. **53**: 325. 1914.

tissues of substances having relatively high cryohydric points gave very little added resistance even though their molecular concentrations were high, while the introduction of substances with very low cryohydric points afforded much added resistance, even at fairly low concentrations. He concluded that since the low-temperature death-point can be lowered by the introduction of substances of low cryohydric points, protoplasm can have no specific death-point, but that the death-point depends upon the temperature at which water and other substances are crystallized out. Some interesting experiments by Gassner and Grimme[25] also show that Maximow's results have a wide application.

The part played by enzymes in plants injured by low temperatures is still rather uncertain, though they are probably involved in the many protoplasmic changes that result. It seems very likely, too, that some of the harmful changes that are caused by low temperatures are due to the perverted action of enzymes no longer properly controlled by substances that have been modified by the cold. Krasnosselsky[26] found that an oxidizing enzyme evinced more activity in sap expressed from a frozen plant than in that obtained from living tissues. The browning of sap expressed from tissues injured by cold is suggestive of the brown-spotting of herbaceous plants obtained by Molisch in experiments cited above, in which low temperatures above the freezing point were used. Möbius[27] obtained very similar results. At any rate, it has been well established that the best known enzymes present in plants are not destroyed by ordinary low temperatures, for Palladin[28] and his students use low temperatures to kill tissues before extracting enzymes. Kovchoff[29] maintains that protein-splitting enzymes are very active in cold-injured plant tissues, though his experiments seem to admit the assumption that perhaps the proteins were split as a direct result of the low temperature and that the changes he records were mainly due to the subsequent increase in the activity of the oxidizing enzyme present in the injured tissues. From his experiments with low temperatures, Schaffnit[30] holds that temperatures a little above the freezing point induce chemical changes in the protoplasm that convert labile into more stable compounds.

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[25] Gassner, G., und Grimme, C., Beiträge zur Frage der Frosthärte der Getreidepflanzen, Bericht. Deutsch. Bot. Ges. **31**: 507. 1913.

[26] Krasnosselsky, T., Bildung der Atmungsenzyme in verletzten Pflanzen, Bericht. Deutsch. Bot. Ges. **23**: 142. 1905.

[27] Möbius, M., Die Erkältung der Pflanzen, Bericht. Deutsch. Bot. Ges. **25**: 67. 1907.

[28] Palladin, W., Über den verschiedenen Ursprung der während der Atmung der Pflanzen ausgeschiedene Kohlensäure, Bericht. Deutsch. Bot. Ges. **23**: 240. 1905.

[29] Kovchoff, J., Enzymatische Eiweisszersetzung in erfrorenen Pflanzen, Bericht. Deutsch. Bot. Ges. **25**: 473. 1907.

The discoloration noted in the new cambium of Fig. 31, Plate XXIV, and in the new wood shown in Figs. 39 and 42, Plate XXV, is probably due to the diffusion into the new tissues of some injurious by-products from cells affected by the cold. This aftermath of low temperature effects seems to account for the fact that bark-injured trees may be sick for some time before they die or recover, and that some may remain dwarfed for years.[31] Münch[32] found that the discoloration so commonly present in the heart wood of some trees is not due to substances secreted by living cells, but to oxidation products arising in dead cells. The presence of fungi increases the extent of the browning. Goethe[33] has also made notes on this diffusion into living tissues of an injurious substance from tissues injured by low temperatures. He found that even in cases of severe bark-injury on the lower portions of tree-trunks, the affected trees survived if this discolorizing substance did not diffuse throughout the sapwood, while if its diffusion was rapid and extensive the tree usually died in a fairly short time. The same facts were found to hold regarding branches at the crotches of which bark-injury had occurred. Sorauer[34] thinks it likely that the injurious substance which diffuses from dead protoplasm into surrounding cells is an enzyme which arose from the disintegrating protoplasm. Active growth is said to check this diffusion or to make it harmless. Bailey[35] found, also, that oxidizing enzymes are largely responsible for discolorations developing in new green lumber during warm, moist weather.

[30] Schaffnit, E., Studien über den Einfluss niederer Temperaturen auf die pflanzliche Zelle, Mitth. Kaiser. Wilh. Inst. Landw. Bromberg **3**: 93. 1910.

[31] Gutzeit, E., Dauernde Wachstumshemmung bei Kulturpflanzen nach vorübergehender Kälteeinwirkung, Arbeit. Biol. Anstalt Lands. Forstwirts. **5**: 449. 1907.

[32] Münch, E., Über krankhafte Kernbildung, Naturw. Zeit. Forst-Landw. **8**: 533; 553. 1910.

[33] Goethe, R., Die Frostschäden der Obstbäume und ihre Verhütung. Berlin. 1883.

[34] Sorauer, P., Was bringen wir mit den Samenrüben und Samenknäueln der Zuckerrüben in den Boden? Zeit. Pflanzenkr. **24**: 449. 1915.

[35] Bailey, I. W., Oxidizing Enzymes and Their Relation to Sap Stain in Lumber, Bot. Gaz. **50**: 142. 1910.

It seems possible, too, that certain degrees of severity in the environment disturb the equilibrium between the enzymes in cells that are in a susceptible condition, and thus eventually lead to disintegration which may culminate in the death of the tissues. Such an assumption might lead to the surmise that the disintegrations evident in the cambial region shown in Fig. 31 are due to an excess of a hydrolyzing enzyme or to the absence of factors that normally inhibit hydrolytic action at a certain stage of growth, and permit the usual maturing processes to go on to completion. Lepeschkin's[36] studies of the effects of high temperatures on protoplasm, as well as some of the results noted by Overton[37] when using heat to kill portions of Cyperus stems, are interesting in this connection because they suggest the possibility that opposite extremes of temperatures may, after all, have some parallel effects.

Although the researches that have been cited on the chemical and physiological phases of low-temperature injury are apparently of fundamental importance, they give only a very meager understanding of what seems to be a small portion of the process. As already mentioned, some of the simpler physical effects of a lowering of the temperature must also be brought into proper relation with the physiological changes induced. After these simpler matters have been disposed of and a fair understanding of the development of bark injury has been attained, the practical phases of the problem will still be unsolved. One who has given this subject much thought cannot avoid the striking fact that *in nature* these injuries ordinarily occur not so much on account of the *degree* of the low temperature reached, as because of the *condition of the bark* at the time of its occurrence.

Some Other Bark Diseases Resulting from Internal Bark Injuries

In the course of my study of crown-rot some other bark diseases were also traced to their origin in bark injuries very similar to those often giving rise to crown-rot. The so-called "cankers," "sun-scorch," and the premature roughening of bark on smooth-barked apple and pear trees were the types most commonly encountered. The latter type of injury was studied by Sorauer[38] about twenty-five years ago. By comparing Fig. 29, Plate XXIV (copied from Sorauer), with Fig. 2, Plate XXI, it is evident that the initial injuries were very similar in the two cases, except that that shown in Fig. 2 is much more severe. Sorauer found that this premature roughening is of especially frequent occurrence on rapidly growing varieties of fruit trees when they are from six to eight years old. The same cold spell that resulted in the bark-roughening described by Sorauer had also caused the bark of some trees to rupture and of others to "scald" or die to the wood in long patches. Some cambium, medullary rays, protoxylem, and pith tissues were killed and discolored; in the cortex the larger patches of dead collenchyma cells were subsequently cut off by new phellogen.

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[36] Lepeschkin, W. W., Zur Kenntnis der Einwirkung supramaximaler Temperaturen auf die Pflanze, Bericht. Deutsch. Bot. Ges. **30**: 703. 1913.

[37] Overton, J. B., Studies on the Relation of the Living Cells to Transpiration and Sap-flow in Cyperus, Bot. Gaz., **51**: 28; 102. 1911.

This type of bark and twig injury of pear trees was apparently also studied histologically by Miczynski.[39] He shows the distribution of dead and discolored tissues in a colored plate. In cases in which the cambium had been killed, the new cambium developed in the inner phloem much like that described in a former section of this paper. Another good but general account of bark injuries of fruit trees is given by Oberdieck.[40] He gives many clear details regarding numerous cases.

Sun-scorch is usually confined to trees that have not yet reached the rough-bark age, and consist of dead and discolored bark on the trunk or main branches, usually (though not always) on the west or southwest sides. Histologically its early stages are similar to those giving rise to the premature bark-roughening described by Sorauer. In many cases of the sun-scorch type, however, only the outermost collenchyma cells are involved, and consequently the resulting new bark surface looks only slightly frayed. Numerous interesting observations have been made on this bark disease, and in many of the discussions one may find pertinent suggestions. Hess,[41] for instance, notes that this trouble develops on smooth-barked forest trees one or more years after the forest has been thinned. Hartig[42] gives some interesting data along this line and concludes that the injury results from the contraction and expansion of the bark rather than from the heating of the sun as is maintained by many, because he found frequent cases of it on north slopes and on the east and north sides of trunks.

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[38] Sorauer, P., Über Frostschorf an Apfel- und Birnstämmen, Zeit. Pflanzenkr **1**: 137. 1891.

[39] Miczynski, K., Ueber das Erfrieren der Gewebe des Birnbaums, Bot. Centralbl. **48**: 228. 1891.

[40] Oberdieck, J. G. C., Beobachtungen über Erfrieren vieler Gewächse und namentlich unserer Obstbäume in kalten Wintern; nebst Erörterung der Mittel durch welche Frostschaden möglichst verhütet werden kann, pp. 108. Ravensburg. 1872.

[41] Hess, R., Der Forstschutz. Leipzig. 1878.

Many of the sections used in the present histological study of crown-rot were made from the bases of shoots arising from large branches of apple trees that had been pruned rather severely, and they therefore also represent the initial injuries preceding the development of crotch cankers as more fully discussed on pages 40–42 of my paper written in 1912. Goethe published a paper in 1877, in which he announced the conclusion that cankers are due to low-temperature injury of the bark. When it was pointed out to him that in Italy where the winters are mild cankers are equally prevalent, he reinvestigated[43] the matter and revised his conclusions to the effect that many of the cankers are due to fungus parasites. It should be noted, however, that his revised conclusion was based largely on the fact that in the spring of 1878 he found new cankers even though no *late* frosts had occurred. (The notion that only late frosts cause these injuries has led many astray.) Fungi developed on cankers when placed in moist chambers, but when spores were used on uninjured bark no cankers resulted. In the following winter bark injuries were numerous in crotches and other places where cankers usually occur. Many of the wounds were carefully cut out in April and most of them healed rapidly, although in a few instances the branches involved died.

Some years later, also, Goethe[44] made an extended study of winter-injuries, giving particular attention to the aftermath or the results of such injuries. A drop in mid October to − 2.5° C. and one to − 10° C. in November very severely injured the pith and other tissues in shoots and the bark of trunks just above the ground. High-headed trees were found more subject to trunk injury than low-headed ones. This is in agreement with what I found in western New York (Tech. Bull. 23, pp. 18–20). Goethe described interesting cases in which the different buds and nodes on the same branch varied greatly in their susceptibility to injury; some remaining normal while others were entirely killed.

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[42] Hartig, R., Ueber den Sonnebrand oder die Sonnenrisse der Waldbäume, Untersuch. Forstbot. Inst. München **1**: 141. 1880.

[43] Goethe, R., Mittheilungen über den Krebs der Apfelbäume. Leipzig. 1877.
——, Weitere Mittheilungen über den Krebs der Apfelbäume, Landw. Jahrb. **9**: 837. 1880.

[44] Goethe, R., Die Frostschäden der Obstbäume und ihre Verhütung, Nach den Erfahrungen des Winters 1879–80, dargestellt. Berlin. 1883.

Müller-Diemits and Stormer-Halle[45] found that fruit trees are most subject to bark diseases at the age when they first become profitable. The bark at the crown, crotches, and various other places on trunks and branches, according to these authors, dies; fungi and bacteria enter the wounds and induce further injury and decay. The wood becomes discolored, and the branch or tree involved dies.

As an illustration of the especial susceptibility of trees to bark injuries and the resulting diseases, during certain stages in their life history, especial attention may be called to the bark-roughening discussed above as well as to this paper by Müller-Diemitz and Stormer-Halle. The older literature of forestry contains many items of interest in this connection. Graebner,[46] for example, described a case of this kind, and in the writings of Hartig, Nördlinger, Hess, and others, are to be found many further instances. Graebner found that a high percentage of trees in a spruce forest had sustained bark injury on their trunks. Very many of them died of crown-rot. The trees had apparently been from 34 to 57 years old at the time the injuries occurred. In an adjoining spruce forest, where the trees were under 20 years of age, no bark injury could be found. It appears from various published statements that spruce trees in a forest stand usually become rough-barked between the ages of 30 and 40, depending upon the rate of growth and thickness of stand.

Conclusions

The histological study here briefly reported, in connection with my two former papers, throws enough light on the earlier stages of crown-rot to permit more definite and general statements regarding its development. It is shown that this and some related bark diseases are not due primarily to the organisms usually found in such affected bark in summer, but to injuries arising when adverse environmental conditions overtake trees having immature bark in certain regions. The rotting of the dead or dying bark is due chiefly to fungi which in some cases also kill living portions while vegetating in severely injured bark.

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[45] Müller-Diemitz, J., und Stormer-Halle, K., Das Obsthaumsterben, Deutsch. Obstbauzeit. **56**: 81. 1910.

[46] Graebner, P., Beiträge zur Kenntnis nichtparasitärer Pflanzenkrankheiten an forstlichen Gewächsen, Zeit. Forst. Jagdwesen. **38**: 705. 1906.

On Plate XXI are shown some of the main types of injuries often found in bark after unseasonably severe periods. This material was collected before evident growth started in the spring and therefore gives some idea of the actual distribution of the injuries. An examination of these figures makes it appear that injuries are of two types: in Figs. 1, 2, 4 and 8 they are evidenced chiefly by a discoloration and collapse of the affected tissues, whereas in Fig. 3 the injury consists mainly of a tangential rupture with only a few of the groups of discolored cells; in Figs. 5, 6, 7 and 9 there occur combinations of the two types of injury. In the latter cases the tissues along the margins of the ruptures are discolored and collapsed much as they are in Figs. 1 and 2. Unfortunately the sections of the material having a combination of the types of injuries shown in Figs. 1 and 2, or of those in Figs. 2 and 7, turned out to be such poor preparations that no use could be made of them.

Plate XXII shows comparable cases as they appeared about two weeks later. This represents a stage of regeneration growth during which living parenchyma cells surrounding injured or dead regions are actively proliferating into spaces formerly filled by the shriveling masses, and into gaps occasioned by ruptures. Figs. 10 and 12 are especially interesting because dead tissues are compressed into more or less radially arranged plates. The proliferating cells are seen to penetrate many of the dead masses, and apparently make contact with living cells beyond. This rapid early regeneration-growth in injured bark is responsible for the fact that so few injured places result in patches of dead bark.

Practically the final alignment of injured and living tissues, as well as the locations of the new meristematic layers, is shown on Plates XXIII and XXIV. From these figures it is evident that when enough of the cambium and inner phloem are killed to form a fairly thick dead layer only a few, or in some cases over considerable areas no living connections are re-established between the old wood and the bark. In some instances the most severely affected bark died early (Figs. 24, 25 and 26), while in others (Figs. 18, 21, 22, 28, 31 and 32) regeneration went on rapidly and the formation of a considerable amount of new wood and bark resulted (Figs. 18, 22 and 31). There are some in which the outer bark has remained alive but in which very little effective regeneration has resulted (Figs. 23, 30, 32 and 35). In most of the last type of cases the bark died to the margin of the severe injury before mid-summer, so that they appeared like Figs. 24 and 26. In instances like those of Figs. 21, 30 and 31 the results were sometimes most striking, because often a fair beginning had been made on the new growth of wood when suddenly the bark died over large areas. In case such an injury occurred on a small branch or shoot, it usually died outright as shown in Fig. 39*a*.

The transition from stages like those shown in Figs. 23, 32 and 35 to those of Figs. 24, 25, 26, 41, 46, 47 and 51 seems fairly clear. The associated micro-organisms evidently play an important rôle in the decay or disintegration marking the later stages of these bark diseases, and in some types doubtless extend the injured areas by their vegetative activities in the places initially killed (Figs. 27, 30, 33 and 35). Yet, this does not seem to be generally the case. The tree shown in Fig. 50 had lived at least fourteen years after the occurrence of the injury, eventually resulting in "heart rot." It is evident from this figure that the fungus rotting the wood present at the time the initial injury occurred has not progressed far outside the last layers of injured wood. In fact, it appears as though it may have rotted only as much of the wood formed since the occurrence of the injury as had been discolored by the diffusion of disintegration products from the initially killed cells. This diffusion injury is shown in Figs. 39 and 42. Yet, in looking over the figures of Plate XXIV it becomes obvious that once a wound parasite, or even a saprophyte especially adapted to a particular host, gains entrance to such an admixture of dead and regenerated living tissues, some living portions may be killed as a result of the vegetative activities of the fungus.

This record of low-temperature injuries occurring in the bark of fruit trees, and of their subsequent development into bark diseases, is of interest and value independent of the factors that give rise to the initial injuries. The diseases in question are thus traced so much nearer to their first causes. Both the macroscopic and the microscopic appearances of much of the bark affected indicate that excessive tensions are developed during the occurrence of the injuries. It remains to be determined whether or not the tension-injury hypothesis of Sorauer is applicable to low-temperature injury in general, in connection with the physiological disturbances induced by the occurrence of severe weather while some of the bark tissues are in certain stages of immaturity or arrested development. It seems possible that, at least in some cases, the presence in the bark of metabolized foods of insufficient concentration to allow normal growth and maturation is the most significant phase of immaturity; the occurrence of droughts appears to have a significant relation. An adverse period in the environment occurring at such a time stops the further accumulation of the labile components of protoplasm, and a long retention of these elementary constituents, together with the enzymes present, may lead to catabolic processes that eventually result in the death of the tissues involved.

ACKNOWLEDGMENTS

In this connection it is a pleasure to acknowledge the aid received from Professors J. B. Overton, L. R. Jones, and E. M. Gilbert, of the University of Wisconsin, during the winter of 1911–12, and especially that of Professor C. E. Allen, of the same institution, for the many excellent suggestions he made regarding the revision of this paper.

DESCRIPTION OF PLATES XXI–XXVII

PLATE XXI

Sections of material collected before growth started in the spring, showing types of initial injuries. All from apple excepting Figs. 1 and 2, which are from pear.

FIG. 1. Injury mainly in cambium, phellogen, and phloem regions; scattered cell-groups in xylem and cortex affected, as indicated by discolored places.

FIG. 2. Another section with severe initial injuries confined chiefly to inner cortex and outer phloem; phloem rays and cambial zone also injured. Scattered groups of dead cells in other parts of cortex and phloem.

FIG. 3. Section of apple branch with common type of injury not usually accompanied by much discoloration, consisting principally of a rupture in the inner phloem and only secondarily of groups of dead, discolored cells.

FIG. 4. Section of apple with most of the injury in the inner phloem, as indicated by discolored streaks and masses. On the left the cambium is killed; on the right it is alive and apparently normal.

FIG. 5. A condition comparable with that of Fig. 4, excepting that a portion of the injury consists of ruptures as shown near the right.

FIG. 6. Some living cambium on the right, and a zone of severe injury in the inner phloem above; on the left most of the cambium is killed. The rays are more severely affected than in the section shown in Fig. 5.

FIG. 7. Collapsed tissue in the inner phloem and cambium is interspersed with a few living cells. Phloem rays are dead, and scattered groups of dead cells occur in the older portions of the phloem.

FIG. 8. Some groups of dead cells and some ruptures occur in the cambium; the inner phloem has but few affected cells. The middle and outer phloem are considerably injured.

FIG. 9. Much like Fig. 7, but with larger groups of living cells in the cambium; more definite radial ruptures are evident in the inner phloem. On the right all of the phloem and cambium between the clefts and the wood are dead.

PLATE XXII

Displacement of initially killed tissues by regeneration-growth. Collected two weeks after the material used for Plate XXI; all from apple.

FIG. 10. A development from an injury like that shown in Figs. 6 and 7. All living cells have grown and compressed the dead material. Living cells proliferate outgrowths into the dead masses. Regeneration tissue is all parenchymatous; it divides in all planes.

FIG. 11. Similar to Fig. 10; in neither of these instances are living connections evident between the bark and wood.

FIG. 12. Different from the two preceding chiefly because some of the cambium has survived. Even where the cambium is killed, frequent living cells are found to bridge the gap. Regeneration has repaired many holes that resulted from the shrinking of groups of dead cells.

FIG. 13. Seems to be a later stage of an initial injury of the type show in Fig. 6; both at the right, where the cambium is involved, and at the left, where the inner phloem is affected, proliferating cells connect the wood with the bark through mantle of dead tissue.

FIG. 14. Though the old cambium (*oc*) was only slightly injured, a new one has begun to develop (*cc*) in the regeneration tissue of the inner phloem. Initial injury in the older phloem was very severe, yet the living portions of it are compressing the dead masses by growth.

FIG. 15. From the type shown in Fig. 8; new cambium (*cc*) is forming in regenerated tissue of the inner phloem, and the former cambial line is discolored. The outer phloem and cortex are almost wholly isolated from the inner bark by a ragged layer of dead tissue. Occasional living cells occur in the dead mass.

FIG. 16. Derived from a portion that was less injured than that shown in Fig. 6. Living tissues dominate, and new cambium (*cc*) has become established.

FIG. 17. Much like Fig. 15, excepting that the injury is much more severe in the old cambium.

PLATE XXIII

Final disposition of much of the initially killed tissue. Collected a month after that shown in Plate XXII; all from apple.

FIG. 18. Late stage of a type shown in Figs. 7 and 9. Former position of the cambium (*oc*) and its present location (*nc*). A considerable layer of new wood (*nw*) has developed, and a new phloem (*np*) is also noticeable. In the phloem the dead masses are localized near the boundary between the old and the new phloem, some extending into the new phloem toward the cambium just as dead streaks extend into the new wood from the injury outside of the old wood (*ow*).

FIG. 19. From the middle toward the right of the figure the new cambium (*nc*) is only faintly indicated. Injury at the old cambium (*oc*) is not as conspicuous as in Fig. 18, but it is of wider extent. Initial injury occurred throughout the old phloem and was very severe.

FIG. 20. Mantle of greatest injury with irregular course. At the right the new cambium (*nc*) has begun the development of a new layer of wood (*nw*), while at the left no substitute cambium is yet visible.

FIG. 21. More irregularity in the course of the mantle of greatest initial injury. Living phloem is left attached to the old wood (*ow*) and converted into wood without leaving an active cambium. New cambium (*nc*) has developed outside the zone of injury that produced the layer of wood. The cambium has become abnormal, yellowish in color, and is partly disorganized. Discolored streaks extend from it into both new wood (*nw*) and new phloem (*np*).

FIG. 22. Much like Fig. 18, showing in addition a new phellogen (*ph*) cutting off the outer part of the cortex.

FIG. 23. Exaggerated form shown near the left end of Fig. 20. No new cambium is in evidence, and dead matter predominates, although from the outside the bark appeared normal.

FIG. 24. A case in which the most severely injured portions of the bark died and a callus (*cal*) developed along its margin. New wood (*nw*) at the lower right arose without leaving cambium.

FIG. 25. Severe initial injury confined to a small space. Although much isolated from the old wood, the callus is normal, having an active cambium. As in Fig. 24, fungus mycelium is present in the dead bark and in the dead mantle between the callus and the dead wood.

FIG. 26. Bark half-way around stem is dead and sunken, much like the patch shown in Fig. 41. In cross-section this looked like a miniature of the specimen shown in Fig. 50. Mycelium and pycnidia of a bark fungus were present.

PLATE XXIV

Higher power views of some injured tissues in the stage shown on Plate XXIII.

FIG. 27. View of a region like that in *oc* of Fig. 18. Living connections through the dead region are evidently few and imperfect.

FIG. 28. Like Fig. 27. Former phloem rays have become discontinued and have undergone division and become converted into callus tissue.

FIG. 29. Copied from Sorauer's paper on "Frostschorf" of apple and pear in *Zeitschrift für Pflanzenkrankheiten*, 1: 137–45. 1891. Cortical injury that usually precedes premature bark-roughening.

FIG. 30. Magnified view of the type shown in the center and right of Fig. 19; substitute cambium (*nc*) developed in a meandering course. Much-injured bark practically isolated from the old wood (*ow*).

FIG. 31. Detail of a case something like that shown in Fig. 22, excepting that practically no new phloem has yet developed; new cambium (*nc*) is considerably disorganized and discolored. Old phloem (*op*) is permeated by initially killed tissue, in direct contact with disintegrating new cambium.

FIG. 32. Much like Fig. 30; only a faint indication of substitute cambium (*nc*) is in evidence. Injury in old phloem is more severe than in Fig. 30; cells in regeneration-growth are less affected by pressure than those in Fig. 30.

FIG. 33. Higher power view of case like that in left-hand portion of Fig. 20, with uncommonly thick mantle of dead tissue. Living portions of former bark rays are converted into ordinary parenchyma.

FIG. 34. Cross-section of a large dead streak of phloem surrounded by modified irregular parenchyma.

FIG. 35. Similar view of a living streak in the phloem surrounded by layers of dead, collapsed cells.

PLATE XXV

Initial injuries followed by another type of regeneration. All from maple except Fig. 41, which is from pear.

FIG. 36. Series of magnified views of a portion of Fig. 37, from old bark (*ob*) to old wood (*ow*): *a*, old bark (*ob*) run through by rifts, new bark (*nb* 1) with inclusions of dead masses, and new cambium (*nc* 1) just outside some new wood shown at the outer edge of outer new wood (*nw* 1) in Fig. 37; *b*, from the inner edge of the outer new wood (*nw* 1) of Fig. 37, showing the new cambium (*nc* 2) and some very irregular new bark (*nb* 2); *d*, from the outer edge of the inner sheath of new wood (*nw* 2) of Fig. 37, *nb* 2 and *nb* 3 together constituting the compressed new bark between *nw* 1 and *nw* 2 of Fig. 37. *e*, higher power view of line *oc* of Fig. 37, showing some detail.

FIG. 37. Cross-section of maple tree (*Acer platanoides*) with a season's growth added after the occurrence of the initial injury, that had been similar to that shown in Fig. 3, and somewhat like that shown in Fig. 38. Three cambial layers have developed in place of one. The tangential cleft left some living phloem adhering to the old wood like that shown in Fig. 3. Substitute cambium arose in the strip of inner phloem adhering to the wood, giving rise to *c* 3 of Fig. 37; then along the inner surface of the loosened outer bark another cambium developed which gave rise to new wood in its *middle* and was thus divided into two cambial sheaths (*c* 1 and *c* 2), each producing wood and bark. Activity of three cambial layers, as detailed in Fig. 36, gives rise to unsightly enlargements like that shown in Fig. 40.

FIG. 38. Section of box-elder tree (*Acer Negundo*) with portion of its bark separated from the wood, though still living. Beginning of callus formation is shown along the edges of the loose bark (May 28).

FIG. 39. Higher power view of a portion shown in Fig. 26*e*; considerable regeneration-growth of wood outside the zone of initial injury, which subsequently died and became discolored.

FIG. 40. Trunk of a street tree (*Acer platanoides*) unduly enlarged near the upper part of the trunk owing to the activity of three cambial zones developed after the occurrence of some injuries initially much like those shown in Fig. 3.

FIG. 41. Trunk of a smooth-barked pear tree in early summer, with a sunken patch over the places sustaining most severe internal injuries.

FIG. 42. Detail view of a section taken across the faintest portion of the line *oc* in Fig. 37, showing that normal new wood (*nw*), arising outside such a line of initial injury, may subsequently be killed and discolored.

PLATE XXVI

Collection of bark-injured and crown-rotted stems, in which the injury was accompanied by radial clefts. All of apple except Fig. 48, which is of orange.

FIG. 43. Apple tree with nearly complete girdle of loose bark (one patch opposite) and a radial cleft 17 cm. long.

FIG. 44. Shows the extent of the loose bark of Fig. 43.

FIG. 45. Twelve-year-old apple tree which had a complete girdle of loose bark from the ground up to the main branches. A radial cleft 25 cm. long occurred in it near ground.

FIG. 46. Apple tree with complete girdle of dead bark; thick callus along its upper edge.

FIG. 47. A typical case of crown-rot on apple.

FIG. 48. Stem of orange tree showing radial clefts in loose bark. Initial injury occurred on the night of November 20, 1914, when the temperature sank to a little below − 2° C. In the summer of 1915 many trees affected in this manner died with symptoms of "withertip."

PLATE XXVII

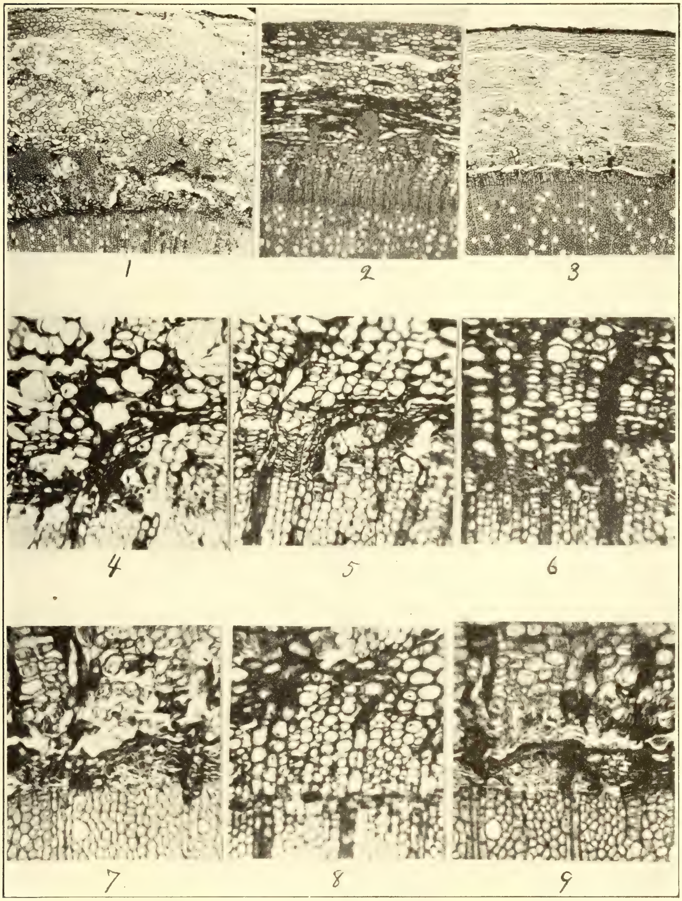
Crown-rot and other troubles of large trees.

FIG. 49. Section from near the base of a large apple-tree trunk (28 cm. in diameter), showing a line of initial injury that occurred some fourteen years before cutting; also showing that the bark sustained a radial cleft (upper side).

FIG. 50. Section of another tree of the same size and from the same orchard as that shown in Fig. 49. The initial injury occurred in the same year as that in Fig. 49. The wood cylinder subsequently died and rotted, and some of the wood produced by the new cambium also decayed.

FIG. 51. Large apple tree with complete crown-rot girdle. Upper roots died, but those under the center of the tree were alive.

FIG. 52. Section of a spruce stem, copied from Hartig (*Untersuch. Forstbot. Inst. München* 1: 147. 1880). Included here to show that the initial injury from which the trouble developed occurred during the dormant season and not during the growing season as was maintained by Hartig.



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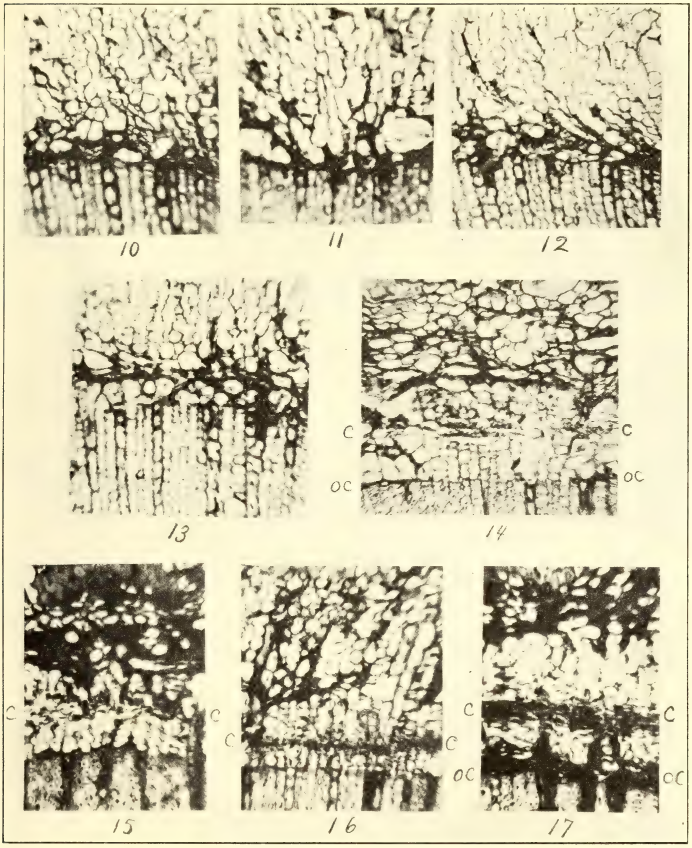
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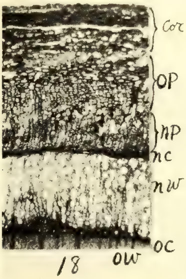
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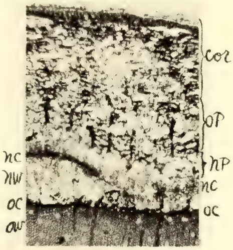
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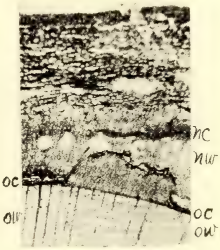
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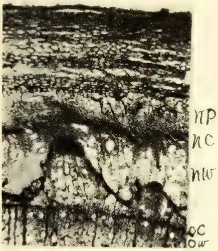
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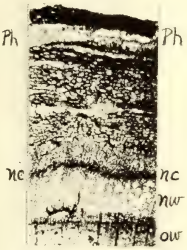
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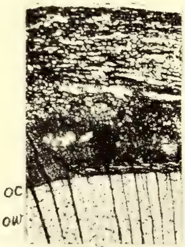

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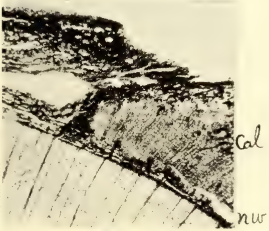

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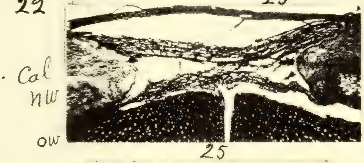

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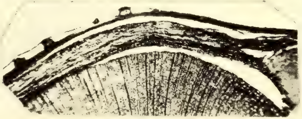

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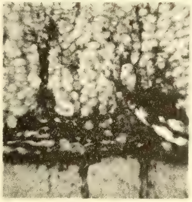

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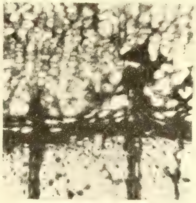

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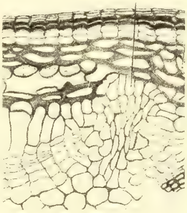

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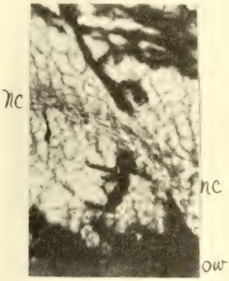

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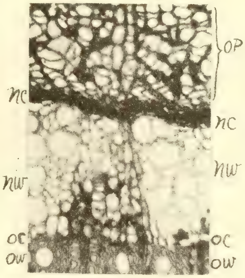
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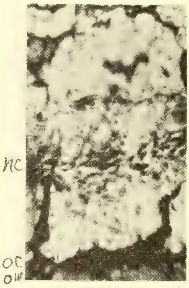

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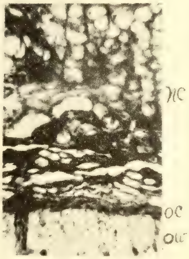

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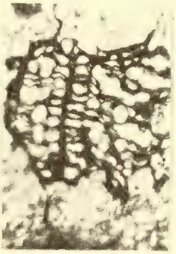

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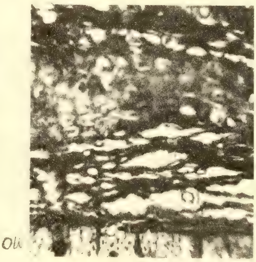

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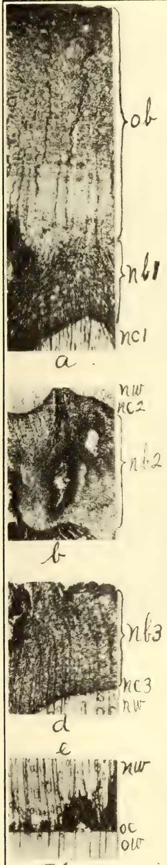
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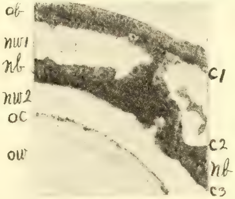
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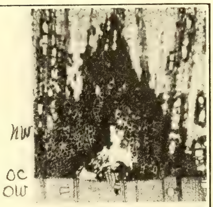

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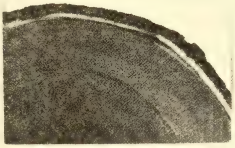

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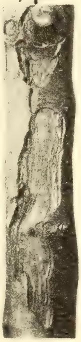

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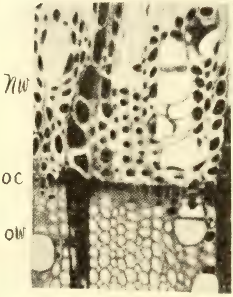

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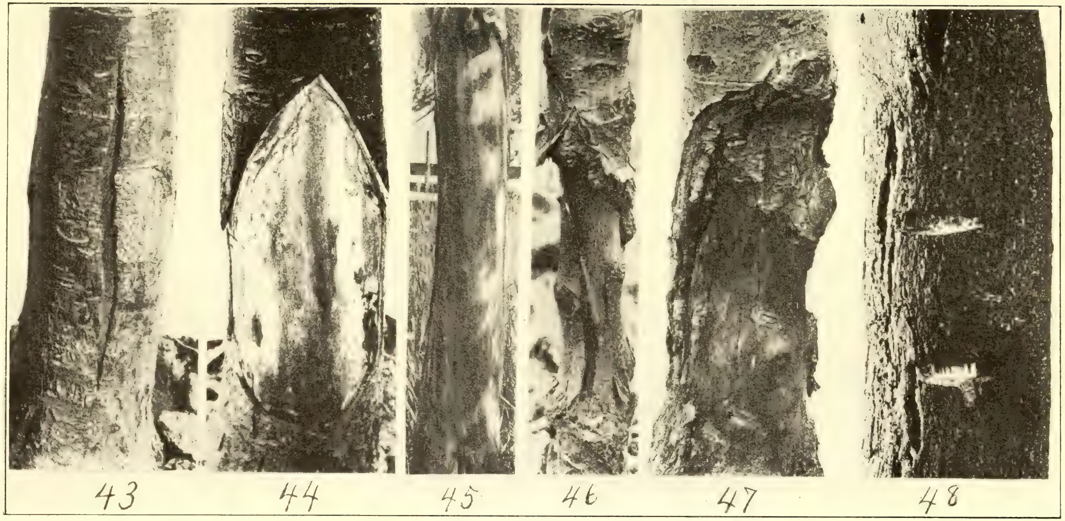
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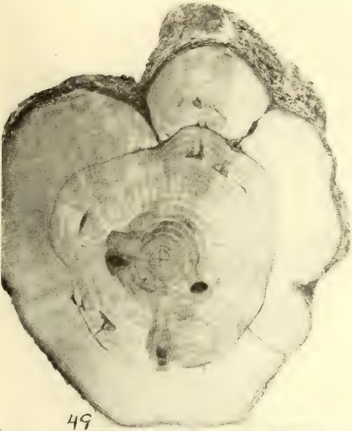

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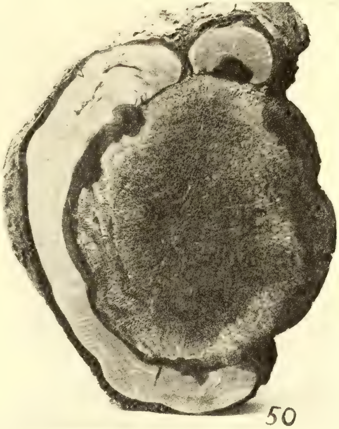

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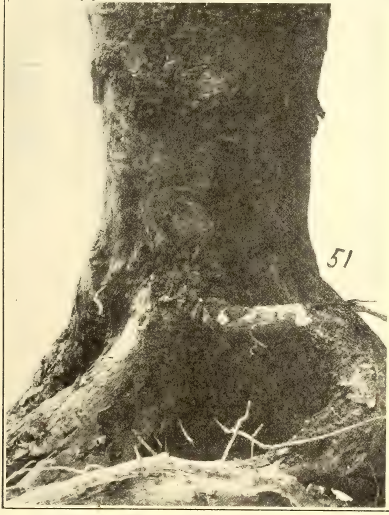

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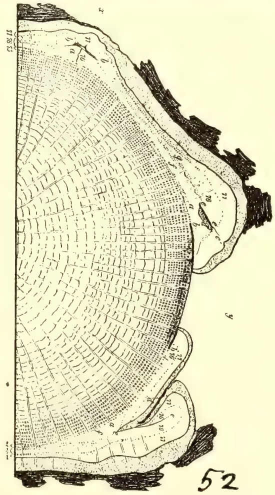

52

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Vol. IV. NOVEMBER, 1917 No. 9

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CONTENTS

Effect of soil temperature on the growth of bean plants and upon their susceptibility to a root parasite..........DONALD REDDICK 513
The development of Cortinarius pholideus..........W. H. SAWYER, JR. 520
Leaf-structure as related to environment..........HERBERT C. HANSON 533

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EFFECT OF SOIL TEMPERATURE ON THE GROWTH OF BEAN PLANTS AND ON THEIR SUSCEPTIBILITY TO A ROOT PARASITE

DONALD REDDICK

The general opinion prevails that temperature plays an important rôle in the infection of a host by a fungous parasite. The experimental data showing just what this rôle is, however, are very meager. In the case of infection of aerial parts other factors are interrelated with temperature, such as persistence of moisture for spore germination, rapidity of germination of spores, and so forth, but in the case of infection of roots by organisms persisting in the soil these conditions ordinarily do not enter. Apparently the soil-inhabiting parasites are largely capable of saprophytic existence so that, given the requisite amount of soil moisture to maintain plant development, the parasite is able to grow and reach the roots of a susceptible host. Gilman[1] has recorded observations on the relation of infection by *Fusarium conglutinans* Wr. on cabbage to soil temperature conditions and thinks a high soil temperature favorable to infection. Gilman[1] continued this work with *F. conglutinans* and appears to have established the point just mentioned, although the control of conditions in some of his experiments was not all that might be wished for. Tisdale[2] arrives at similar conclusions in connection with the infection of flax (*Linum usitatissimum*) by *Fusarium Lini* Bolley and states that the low critical temperature is about 15°–16° C.

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[1] Gilman, J. C. The relation of temperature to the infection of cabbage by *Fusarium conglutinans* Wollenw. (Abstract.) Phytopathology **4**: 404. 1914. Cabbage yellows and the relation of temperature to its occurrence. Ann. Mo. Bot. Gard. **3**: 25–82. 1916.

[2] Tisdale, W. H. Relation of temperature to the growth and infecting power of *Fusarium Lini*. Phytopathology **7**: 356–360. 1917.

[The **Journal** for October (**4**: 439–512) was issued October 2, 1917.]

Smithsonian Institution
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National Museum

The writer undertook an investigation of somewhat similar nature, using the bean, *Phaseolus vulgaris*, as host and *Fusarium martii phaseoli*[3] for the parasite, a fungus that has been shown by Burkholder[3] to be the cause of a serious disease of beans in New York.

The work was performed in the laboratory of plant physiology of the Johns Hopkins University, Baltimore, Maryland, where the writer was fellow by courtesy during the academic year 1916–17. His thanks are due the authorities of that institution for the facilities afforded him and special acknowledgment is made of the critical advice given him by Prof. B. E. Livingston, under whose immediate guidance the work was done. While the investigations are by no means completed, some of the physiological features of the results thus far obtained are of sufficient interest to warrant this note.

The plants were grown in cylindrical vessels of tinned sheet-iron, 17 cm. tall and 15 cm. in diameter, which in turn were placed in a water bath. The garden soil used was first heated in an autoclave for one hour at a temperature of 110° C. and it was then made uniform by repeated sifting. The culture vessels were filled and nearly uniform packing was obtained by letting the soil fall into place always from the same height. Water was supplied by means of the Livingston auto-irrigator,[4] two cylindrical porous clay cups being used, each with an exposure to the surrounding soil of approximately 121 square centimeters.

The irrigation water was drawn directly from the water of the bath and care was taken to have the supply uniform, so as to avoid difference in soil moisture content that might influence the growth of the plants.

Since the water level was nearly as high outside the cylinders as was the level of the soil within, it was necessary, while the plants were small, to retard the flow of water into the cups. This was accomplished by introducing mercury columns of equal heights into all the supply tubes. Later the mercury was not needed and was removed.

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[3] Burkholder, W. H. Some root diseases of the bean. (Abstract.) Phytopathology **6**: 104. 1916. Bean diseases in New York State in 1916. (Abstract.) Phytopathology **7**: 61. 1917.

Burkholder states that *Fusarium martii* Ap. & Wr. does not produce infection on the bean but that the fungus from bean is nearly identical with this species. The name *martii phaseoli* has not been used previously and is only introduced here as a matter of convenience.

[4] Livingston, B. E. A method for controlling plant moisture. Plant World **11**: 39–40. 1908.

Hawkins, Lon A. The porous clay cup for automatic watering of plants. Plant World **13**: 220–227. 1910.

Livingston, B. E., and Hawkins, Lon A. The water-relation between plant and soil. Carnegie Inst. Wash. Publ. **204**: 3–48. 1915.

The water baths employed were three in number, each 60 cm. in diameter and 25 cm. deep (ordinary galvanized iron laundry tubs) thus giving space for seven culture vessels each. A wood grating at the bottom supported the culture vessels and allowed them to be submerged to within one centimeter of the top.

Three temperatures, 34°, 22°, and 15° C., were arbitrarily decided upon, but this choice was governed somewhat by the facilities available. The highest temperature was obtained by means of an electric heater under thermostatic control, and was maintained uniformly throughout the course of the experiment.

The medium temperature followed that of the culture room; there was no special control in this bath. Because of the great bulk of water, the fluctuation in temperature was not very great. The range was from 20° to 23° C. (usually 21° to 22°) whereas the diurnal variation in the temperature of the greenhouse room was large, 12° to 28° C. In our present state of knowledge of the influence of soil temperature on host or parasite this fluctuation is to be regarded as of little consequence but obviously some constant temperature might have been maintained with very little difficulty, by employing such an outfit as was used for the highest temperature.

A constant water level was maintained in each of the two warmer baths by means of a Mariotte flask.

The lowest temperature was obtained by passing a continuous stream of tap water through the bath. When the water flowed at the rate of 1,500 cc. per minute a temperature of 15° was maintained, during the winter months. On very warm days a rise of two or three degrees sometimes occurred. The total range was from 14° to 18°.

The surface of the water in the baths was covered with a thick paraffin oil to reduce loss of heat by evaporation and to eliminate the vapor blanket that would otherwise have been present over such an exposed body of water. Later it was found that a covering of ordinary paraffin (melting point about 50°) was very much better for the purpose. This was melted and poured on the water, where it was allowed to spread and harden.

The soil in four of the culture vessels of each series was contaminated by sprinkling in it, when nearly full, some soil heavily laden with viable spores and mycelium of *Fusarium martii phaseoli* from culture. The fungus, which was supplied through the courtesy of Dr. W. H. Burkholder, had been maintained for several months in pure culture but the medium (bean-pod decoction agar) was uniform throughout the period, and a sub-culture had been made every ten days.

Pure-line seeds of a pea bean,[5] were disinfected externally with a 1 to 1,000 solution of mercuric chlorid, after which they were sprouted in a moist chamber. They were planted on January 10, 1917, six[6] seeds in each culture.

When the cotyledons had broken through the ground all plants were inoculated with *B. radicicola* by injecting into the soil about the roots one cubic centimeter of a heavily laden water suspension of this organism taken from bean nodules, and the number of plants per pot was reduced to four.

After twelve days the plants with soil temperature at 34° were developing the first trifoliate leaf; those at 22° had just spread the first pair of true leaves and those at 15° were not all through the soil surface. On the forty-fifth day the plants at 34° were beginning to blossom while those at 22° began blossoming eleven days later. The plants at 15° were either dead or very poor and none developed satisfactorily. A single one of these cold-soil plants finally reached a height of about 15 cm. and produced one blossom but did not set a pod. It is to be borne in mind that the air temperature here was practically the same as that of the plants with soil temperature of 22°.

Unfortunately some of the plants in the control cultures became infected, the contamination apparently being carried by numerous small insects that were abundant on the plants. In the cultures at 22° five of the twelve plants were diseased and in those at 34° eight of the twelve plants were affected. All of these plants were affected relatively late as compared with the inoculated plants, so that it is impossible to judge what amount of damage may be attributed to the disease, but it was obvious at harvest time that the plants in two of the control cultures of the series of 24° were severely injured.

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[5] The seed was supplied through the courtesy of the Department of Plant Breeding, Cornell University and is maintained under the department number 1986–2.

[6] From the outcome of this experiment and numerous others subsequently performed with beans of this and another pure line, and with beans secured on the open market, it is very evident that not enough seeds were used at the outset. After seeds of uniform size and appearance are selected it is safe to allow for only about 25 percent as likely to yield plants entirely free from defect and of perfectly uniform appearance. Weak plants frequently cannot be detected for ten days or two weeks after the plants emerge from the soil.

In addition there were "weak" plants in nearly every culture. These could not be detected as such for two weeks or more after the plants were up and it was then too late to correct for the trouble. In fact it was thought for some time that some of these plants were ones on which infection had been particularly severe. As there were plants of varying degrees of "weakness" it is not possible to throw the poor plants out of consideration.

Furthermore, it is not possible to make a comparison between the cultures grown at the two temperatures because of the fact that the plants grown at high temperature developed more rapidly from the very beginning and thus matured under a different set of air conditions. In this experiment this meant that the plants grown at the highest temperature had very much less sunshine than those grown at 22°. The difference is noticeable in part in the total dry weight of seed, but some of the difference is attributable to a more severe infection on control plants grown at the high temperature.

Finally the difference in growth at the two different temperatures might have been due in part to a difference in air temperature. Thermometers suspended over the water baths at a distance of 15 cm. from the surface showed constantly a higher temperature over the bath at 34° than over the ones at 22° and 15°. The difference varied from .5° to 4.5° and averaged from 3° to 4° higher.

With these four considerations in mind it may now be stated that the average yield per plant for "healthy" plants in the series at 34° was 1.451 grams of air-dry seed. For the infected plants the average was 1.081 grams. Thus the presence of this Fusarium on the roots of beans under the conditions stated resulted in a direct loss of 25.5 percent. For the cultures grown with a soil temperature of 22° the average yield per healthy plant was 2.361 grams. For the inoculated plants it was 1.557 grams. Here the reduction in yield on account of disease was 34 percent.

The most interesting feature of the experiment is the fact that these beans grew faster and matured a crop earlier with the higher soil temperature. The relatively small difference in air temperature may account for some of this difference in growth but certainly cannot entirely account for the results obtained. Wholly aside from its scientific interest the question may have an important practical bearing for those engaged in the production of flowers and vegetables under glass, and from either standpoint is worthy of further attention.

The idea of supplying bottom heat has been used extensively by florists[7] for starting cuttings, but not for growing crops. Plant physiologists do not seem to have studied the problem, judging by the absence of literature on the subject, but this experiment with beans and some trials with radish (*Raphanus sativus*), cucumber (*Cucumis sativus*), and tomato (*Lycopersicum esculentum*) indicate that root temperature and foliage temperature are readily separable as conditions influencing the growth of plants.

With respect to susceptibility due to environmental changes it would seem that in the case of temperature as applied in this experiment the relation between host and parasite cannot be analyzed readily. The experiments show that the host is influenced markedly by a change in soil temperature so that it is impossible to make a direct comparison of various temperature conditions because of the slow action of the parasite. If the parasite made a rapid invasion and killed the host outright within a few days there would be an opportunity to grow all plants under identical conditions until the day of inoculation but even then the sudden change of soil temperature might have an even more marked effect on the physiological condition of the host, perhaps changing its susceptibility in a very pronounced manner. In the case of this disease, and of the majority of root diseases, prompt death of the host does not follow because some water continues to enter even after the roots have been killed and especially because on most plants new roots generally push forth above the point of infection.

In will be necessary to study under controlled conditions the behavior of the uninfected host when subjected to certain changes in this one environmental condition, and that of the parasite in the same way, in order to determine the true relation of host and parasite. This involves the control of all the known conditions affecting the growth of plants, including light, a method for doing which has only been hinted at[8] to date.

The physiology of the fungus here used has not been the subject of investigation as yet, but in some preliminary experiments on the rate of growth of the fungus at different temperatures it was found that the diameters of the thalli on bean broth agar in petri dishes varied with the temperature. In one instance, at the end of five days, the diameters of the thalli in millimeters for the stated temperatures are shown in the accompanying table.

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[7] White, E. A. The principles of floriculture. p. 162–164. New York. 1915.

[8] Livingston, B. E. Plant World **20**: 11. 1917.

TABLE I

Diameters in Millimeters of Thalli of Fusarium martii phaseoli when Grown for Five Days at the Temperatures Indicated

Temperature °C.	Diameter of Thallus Millimeters
12–13	8
15–16	12
17–18	15
19–21	17
23–25	23
26.5–27.5	28
30.5–31.5	34
35–35.5	13
38.5–39	No growth

It appears from the table that the highest temperature selected for the experiment was one near, but perhaps slightly above, the optimum for the growth of this fungus, but it is to be noted that growth takes place at a temperature much below the lowest temperature selected and infection occurred on inoculated plants in the cultures at all three soil temperatures employed.

It is unfortunate that a low temperature was not selected that would at least have permitted the growth of beans even though poorly. It is well known[9] that beans require a warm soil for their best development. In a cold soil presumably bean plants would not have as great vitality and might have proved particularly susceptible to this hemiparasite. Likewise, in the case of cabbage it is well known that the plants do well in a cool summer and poorly in a warm one. At the higher temperatures the plants may possess a lower degree of vitality and hence should be more susceptible to facultative parasites. This point Gilman passes over lightly in his work.

CORNELL UNIVERSITY,
ITHACA, NEW YORK.

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[9] Reynolds, J. B. Temperature in relation to seed. Ont. Agr. Col. Rept. 29 (1903): 9–11. 1904.

Sevey, Glenn W. Bean Culture, p. 7. New York. 1914.

THE DEVELOPMENT OF CORTINARIUS PHOLIDEUS

W. H. Sawyer, Jr.

Introduction.

Cortinarius pholideus is characterized by the peculiarly strong development of dark, pointed, erect scales on the pileus and stem. This feature is unusual in Cortinarius, but is very striking in certain species of Pholiota, so that *Cortinarius pholideus* in its general aspects suggests Pholiota, the spore color in the two genera being the same. Since I have recently studied three scaly species of Pholiota (12), it therefore occurred to me that it would be extremely interesting to study the development of this species, which I found in all stages of development in the same region in which the Pholiotas[1] were collected. Especially would it be interesting to determine the formation of a cortina in a species where such a prominent, coarse, universal veil is present.

Primordium of the Basidiocarp

The very young fruit-body is elongate, composed of slender, closely interwoven hyphae, with numerous interhyphal spaces. These hyphae are, in general, parallel with the long axis of the basidiocarp; they have abundant protoplasm and are active in growth, as indicated by their deeply staining property and long slender cells. The peripheral threads, however, take the stain poorly or not at all. They turn outward on all sides, and in an extremely early stage the outermost cells are enlarged, dead, and brown in color.

This outer zone of differentiated hyphae forms a loose-meshed envelope for the entire plant, and is a universal veil, or blematogen, in the sense in which this structure has been interpreted by Atkinson (4).

Figure 1 represents a median longitudinal section through a very young fruit-body, which is about one millimeter long and half a millimeter in width. The hyphae are slender and very uniform in size, averaging about 3 μ in diameter. The loose peripheral threads belonging to the blematogen, however, are enlarged, many of them being 10 μ in diameter, dead, and brown. A conspicuous feature of the young fruit-bodies of this species are the numerous interhyphal spaces scattered throughout the basidiocarp.

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[1] Woods in vicinity of Seventh Lake, Adirondacks, N. Y.

DIFFERENTIATION OF THE STEM FUNDAMENT

Very early in the development of the fruit-body the hyphae in the basal part increase in number and show evidence of more rapid growth, so that this portion of the basidiocarp becomes more dense in structure. This new growth is the primordium of the stipe, which, by progressive growth and differentiation, finally reaches the apex of the fruit-body. From the beginning, the structure of the basidiocarp is compact, and the gradual progressive differentiation of the stem fundament does not at first produce any marked change in appearance. In figure 2 the fundament is well differentiated nearly to the stem apex; the latter is still in the primordial condition. In figure 3 at the left is shown the compact cortex of the stem fundament, from which the looser hyphae of the inner portion of the blematogen radiate outward and upward. The cortex of the stem fundament is somewhat more dense and deeply staining than the inner portion, as shown in figure 5.

A similar, but more marked origin of the stem, has been shown to occur in two species of Lepiota (6), in *Rozites gongylophora* (11), in the three species of Pholiota already mentioned, and in five species of Cortinarius (8).

DIFFERENTIATION OF THE PRIMORDIA OF PILEUS AND HYMENOPHORE

After the organization of the stem fundament the hyphae in its apical end take on more rapid growth and branch freely, as indicated by their deeper stain and by the fact that they traverse the interhyphal spaces at this time. This new interstitial growth causes a bulbous expansion of the stem apex, which marks the young primordium of the pileus (fig. 6). At the same time the peripheral hyphae of this apical region, instead of growing, in general, straight upward, as they have done heretofore during the development of the stem fundament, now grow outward in all directions (fig. 7). On the lateral surfaces they become subject to epinastic influence and turn strongly downward, forming the pileus margin, as shown in figure 8 by the small deeply stained area on either side of the pileus, beneath the blematogen layer.

Almost simultaneously with the formation of the pileus margin the hyphae of its under surface begin to grow outward and downward very rapidly. These hyphae are slender, very rich in protoplasm, crowded together, and with terete ends. Their outward growth while under the influence of epinasty causes them to curve strongly, so that the ends point downward, or even inward toward the stem. This ring of new growth surrounding the stem apex is the hymenophore primordium. A median longitudinal section at this stage shows it as a deeply stained region on either side, as in figure 10. Since the primordium of the hymenophore is formed from hyphae of the pileus margin, and at practically the same time with the latter, it is extremely difficult to point to the exact stage at which it originated. As has been stated, the hyphae of the pileus margin stain deeply and by new growth increase the density of its structure, and the beginning of this period of increased activity probably marks the origin of the hymenophore primordium.

The appearance of this new fundament definitely marks off the pileus area from the stem primordium. As development continues, the pileus broadens centrifugally and becomes more compact by interstitial growth. At the same time the hymenophore primordium, by the intercalary growth of new hyphae from the pileus, and by the increase of its own elements, likewise develops centrifugally, and keeps pace in its growth with the pileus margin.

Formation of the Palisade Layer

For a time the growth of the hyphae composing the hymenophore is very rapid and uneven, the pointed ends of some of the threads growing down beyond the others, so that the surface is rough and jagged (figs. 12 and 13). Gradually, however, the hyphae acquire a more uniform rate of growth, and the ends reach the same level, becoming clavate and crowded. This condition of the hymenophore in which the hyphal ends form an even, compact surface is the palisade stage. Such a condition is shown in figure 23. In this species, as in others previously investigated, its development is centrifugal, from the stem toward the pileus margin. Here however it develops very uniformly, so that at one time in the same fruit-body the palisade occupies the whole area of the hymenophore except the extreme margin. In all the species that have had this phase of their development described, the formation of the palisade is more gradual, so that in the same fruit-body there is present at the same time the palisade condition of the hymenophore and a considerable amount of primordial tissue.

The Annular Prelamellar Cavity

When the pileus and stem areas become differentiated from the primordial tissue of the basidiocarp, in the angle formed by the junction of these two structures a small amount of primordial or ground tissue is left. The primordia of pileus, stipe, and hymenophore grow more rapidly than does this ground tissue and as a result tensions are produced which cause it to become loose in texture and to tear apart. This results in the formation of a cavity in the form of a ring around the apex of the stem, beneath the surface of the hymenophore. In this species the formation begins very soon after the origin of the hymenophore, as shown in figures 10 and 11, where the tissue immediately below the hymenophore primordium is becoming loose through the lateral and upward pull exerted on it by the margin of the expanding pileus. In figures 14 and 15 the development has proceeded further, so that an actual cavity is formed, although still weak and spanned by hyphal threads. By the time the level palisade stage is reached the gill cavity is well differentiated and entirely free from intervening tissue, as shown in figures 16, 17 and 23. Like the primordium of the hymenophore and like the palisade, its development is from the stem toward the pileus margin, so that its earliest appearance is close to the stem. The two tangential sections represented by figures 12 and 13 show this fact; in figure 13 the cavity, while not complete, is more strongly developed than in figure 12, a section of the same fruit-body nearer to the pileus margin.

The Origin and Development of the Lamellae

At the time of the beginning of gill formation the pileus and stem are completely formed and the gill cavity is well defined. The hymenophore is in the stage in which there is an even palisade layer extending from the junction of the stem and pileus nearly to the pileus margin; near the latter the palisade grades off into the primordial condition of the hymenophore. The palisade is composed of small hyphae with blunt and crowded ends (fig. 23). The continued growth of these hyphae and the intercalation of new elements from the hymenophore above gives rise to sufficient lateral pressure to throw the palisade surface into downwardly projecting folds (fig. 24) which are the first gill salients. At the same time a more rapid growth of the hymenophore downward in radial, regularly spaced areas directs the formation of the folds, as described for *Agaricus rodmani* (5) and species of Coprinus (7), so that the gills are radially symmetrical with reference to the stipe.

The origin of the lamellae is next the stem, and by continued growth and differentiation the lamellae develop toward the pileus margin. This centrifugal manner of formation enables one to study their development by means of serial longitudinal sections from the pileus margin toward the stem, since they are youngest near the former and become progressively older as they approach the latter. Figure 23 represents a section near the pileus margin. The even palisade occupies the greater part of the hymenophore surface, with a little of the primordial tissue on either side. The gill cavity is well formed. The tissue below the latter belongs to the stem cortex and universal veil, together with some ground tissue belonging to the partial veil. Figure 24 represents a section a little nearer to the stem. The palisade is no longer level, but has an undulating surface, with two slight, very broad folds. In figure 25, still nearer to the stem, these two folds are more pronounced, and at the right the beginning of a third may be noted. The breadth of these folds, and their distance apart, can leave no doubt that they are the first salients of the lamellae themselves.

The trama of the mature gill (fig. 34) has its origin in the hyphae beneath the palisade layer which grow down into the young gill salient. Further growth takes place by the elongation and enlargement of these hyphae. Throughout the center of the lamella they are compactly interwoven, with their general direction of growth toward the edge (fig. 34). Laterally, however, they turn outward and form the hymenial layer of the lamella.

The primary gills, because of their radial arrangement with the stipe as a common center, diverge as they approach the pileus margin. Continued growth of the hymenophore results in the production of shorter secondary lamellae between their outer ends in the same way as that in which they were formed. Figures 28–32, however, show the formation of two gills in a somewhat different manner. In figure 28, a section near the stem, it will be noted that a gill salient occurs that is unusually broad. In the successive sections it can be seen that this broad salient, by branching, forms two salients, each of which thereafter develops into a lamella in the usual way. This probably illustrates the method of origin of the dichotomous or forked lamellae characteristic for Cantharellus and certain species of Russula.

Figures 35–37 illustrate a condition due to the strongly inrolled margin of the pileus. Figure 35 is of a section tangential to the pileus margin. In the center a lamella appears with a cavity on either side. Figures 36 and 37, respectively, nearer the stem, show the same condition; the lamellae appear as bars continuous from the upper to the lower part of the pileus, with separate cavities between them. The gills, however, have not become continuous with the tissue below by growing down and uniting with it. This tissue belongs to the hymenophore of what is morphologically the under surface of the pileus. The inrolling of the margin of the latter, however, has reversed the position of the hymenophore. The presence of the salients of secondary lamellae on this lower surface serves to make this more clear. The attachment of the gills below, as well as above, represents their point of origin. The sections are not cut perpendicular to these points, but are tangential to the "backs" of the lamellae; their direction of growth is not in the plane of the section, but at right angles to it. The spaces between the lamellae are extensions of the general annular cavity nearer the stem.

The Blematogen

Before any internal differentiation takes place the young basidiocarp is completely enveloped by a universal veil or blematogen. The hyphae composing this outer layer are differentiated from the other elements of the fruit-body by the fact that their cells are short and enlarged, the outer ones being dead, with thick brown walls and scanty content. The direction of these hyphae is outward and upward. They diverge at the ends, forming a loose structure easily rubbed off during growth or in the manipulation of the fruit-bodies preparatory to study.

The blematogen has a very striking appearance at about the time of the formation of the hymenophore primordium (fig. 10). The large hyphae stand straight out from the pileus surface, their clear yellow-brown walls, which do not stain at all, contrasting sharply with the deeply stained and closely interwoven elements of the pileus. At this same time the weft of hyphae between the pileus and blematogen, left when the former became differentiated from the primordial tissue, loosens, probably because of partial cessation of growth, and forms a thin layer with many interhyphal spaces extending over the surface of the pileus (figs. 17 and 19). As the plant approaches maturity the erect hyphae of the universal veil (figs. 18, 19) become aggregated into little tufts or clumps that form the erect, dark scales covering stem and pileus, so characteristic of this species.

A peculiar and interesting feature of the blematogen is its double character over the margin of the pileus, as shown in the left side of figure 14 and in figure 15. The outer layer is characteristic of the universal veil elsewhere on the plant, being composed of large, thick-walled cells that radiate outward in loose arrangement. The inner portion, however, is very different in appearance. The hyphae are slender, with abundant protoplasm and thin walls. Instead of growing outward in loose structure they lie closely side by side and passing up over the edge of the pileus margin become ingrown with the pileus surface an appreciable distance above its free edge. Kniep (10) has demonstrated that in hyphal threads bearing clamp connections, the growing end always lies in the direction in which the obtuse angle, formed by the junction of the cross wall of the hypha and the cross wall of the clamp, opens. These inner blematogen hyphae bear numerous clamp connections, whose walls all form angles opening upward; therefore these hyphae could not have grown down from the pileus, but must have had an upward direction of growth. Furthermore, in the section shown in figure 16, the free ends of some of these hyphae may be seen interlacing with the threads of the pileus surface just above the margin of the latter. It is probable that the growth of this inner layer is slow, and its union with the pileus is due to the active outward and downward growth of the hyphae belonging to the latter, which interweave with the threads of the former. A duplex blematogen has been described by Miss Douglas in *Cortinarius anfractus* and *C. armillatus* (8), differing, however, from the condition here in that the outer layer in these two species is thin and compact, while the inner part is loose and floccose.

The Marginal Veil

The marginal veil is very poorly developed in this species, as compared with *Agaricus rodmani* (5), *Armillaria mellea* (3), *Agaricus comtulus* (2), species of Hypholoma (1), and other species. After the differentiation of pileus and stem, some ground tissue is left in the angle between them. This is nearly all broken away in the process of formation of the gill cavity, but a small amount may remain attached to the pileus margin, beneath the blematogen. This is increased by the downward growth of a few hyphae from the extreme margin of the pileus, and in figures 14 and 15 it is probable that the inner layer of the veil described above does not belong entirely to the blematogen, but has on its inner surface some hyphae belonging to the marginal veil proper, as limited from the universal veil or blematogen.

The Cortina

The name "cortina" is a term applied especially in the genus Cortinarius to the veil composed of delicate silky fibrils stretching from the pileus margin to the stem. It is usually evanescent, although in a few species, like *C. armillatus*, it may persist for a long time in the form of rings about the stem. In *C. pholideus* it breaks away early, leaving a very slight ochraceous annulus around the top of the stipe that disappears with age. Occasionally a half-grown plant is found with the arachnoid veil still intact. It is light in color, almost white, and stretched tightly over the gills. The fibers composing it are very slender, and this character, together with its lighter color, distinguishes the cortina from the brown-walled, larger hyphae of the outer blematogen layer, which is external to the cortina in varying amount, depending on how much has been rubbed off during the development of the plant. The cortina is composed of the hyphae of the inner layer of the blematogen, together with whatever marginal veil may be present. In figures 20 and 21 it may be seen extending from the pileus margin to the stem. Outside is tissue belonging to the outer layer of the blematogen. Figure 22 shows a condition so common as to be almost characteristic in this species, in which the pileus margin is so strongly inrolled that it has become free from the cortina, which is attached to the pileus surface above its margin, thus showing that the cortina represents here the inner zone of the duplex blematogen.

Fries (9) must have regarded the cortina as a structure distinct from the universal veil or blematogen, for, although its presence was used by him for a generic character, only two of his six subgenera, namely Myxacium and Telamonia, are said to possess a universal veil. *C. pholideus*, however, put by Fries in the sub-genus Inoloma, has a universal veil, and the same has been found by Miss Douglas in species representing two other Friesian subgenera. No generalizations can be made until the development of many more species is known, but the evidence at hand indicates that the presence of a universal veil (blematogen) is constant for the genus. If so, it is probable that it plays some part in the origin of the cortina, as in the species studied by Miss Douglas, and as it does in this species.

In conclusion, I wish to acknowledge my indebtedness to Professor George F. Atkinson, under whose direction the greater part of this work was done at Cornell University, for his helpful interest and kindly criticism.

SUMMARY

1. The primordium of the basidiocarp of *Cortinarius pholideus* is composed of slender hyphae interwoven into a compact structure with numerous interhyphal spaces, and enveloped in a layer of differentiated hyphae.

2. These enveloping, radiating hyphae form the blematogen or universal veil. They are loose in their arrangement, with large, thick-walled cells. Soon after pileus formation the blematogen shows a double character over the pileus margin and gill cavity. The inner layer has an upward growth direction and the hyphae of the pileus surface interlock with its upper portion.

3. The appearance of the stem fundament is the first differentiation to take place within the basidiocarp. It is formed in the base of the fruit-body, and advances to the apex by progressive growth and differentiation.

4. The pileus is formed by the expansion of the stem apex, due to interstitial and divergent growth. The lateral hyphae of the pileus fundament by epinastic growth form the pileus margin.

5. Perpendicular downward growth of hyphae from the under surface of the pileus, beginning in the angle between stem and pileus, forms the primordium of the hymenophore as an annular zone of new growth surrounding the stem apex. At first, because of unequal growth of its hyphae, the primordium is uneven and jagged, but later the ends of the hyphae grow down to the same level, forming the even palisade zone.

6. The annular prelamellar cavity is formed by the breaking away of ground tissue left in the angle between stem and pileus after their differentiation, due to the growth and expansion of these parts. A small amount of this ground tissue may remain attached to the edge of the pileus and form a slight element of the cortina.

7. The lamellae originate as downward-growing folds of the level palisade zone, through the influence of lateral pressure in the palisade, and, more particularly, by downward growth of hyphae from the hymenophore in radial, regularly spaced areas. Their differentiation is centrifugal, from the stem toward the pileus margin. The first folds or ridges in the hymenophore are the salients of the lamellae themselves. The gill trama is formed by the downward growth of hyphae from the hymenophore into the gill salient, and increases by interstitial growth.

8. The cortina is the silky veil stretching over the gills, attached on the one hand to the surface of the pileus margin and on the other to the stem. It is composed of the hyphae of the inner layer of the blematogen, together with fragments of the ground tissue below the hymenophore. It is covered externally by remnants of the outer layer of the blematogen, as indicated by the dark patches that may be attached to its outer surface.

Botanical Department,
Cornell University.

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DESCRIPTION OF PLATES XXVIII–XXIX

The following microphotographs were made by the author, some with the Spencer Lens Co.'s horizontal camera with Zeiss lenses, the others with a Bausch and Lomb microscope equipped with Zeiss lenses and a Bausch and Lomb vertical camera attachment.

Plate XXVIII

Fig. 1. Primordium of the basidiocarp. The hyphae are closely interwoven to form a compact structure. Many interhyphal spaces occur, scattered through the fruit-body. On the outside are a few blematogen hyphae. ×50.

Fig. 2. A fruit-body somewhat older than the preceding. The stem fundament is differentiated to near the apex, where the hyphae are still in the loose primordial condition. On the sides may be seen the basal parts of blematogen hyphae, their outer ends having been lost. ×30.

Fig. 3. An enlargement of the right side of the preceding figure. The loose blematogen threads are shown growing out from the compact stem cortex. ×215.

Fig. 4. A young fruit-body after formation of the stem primordium. The blematogen may be seen enveloping the stem fundament. ×30.

Fig. 5. A slightly older stage, showing the blematogen radiating from the stem cortex. The latter is compact; the apex is slightly more deeply staining, showing that growth is more active at that point. ×30.

Fig. 6. A state slightly older than the one shown in fig. 5. By interstitial growth the stem apex has become bulbous, forming the pileus primordium. Outside is the blematogen. ×50.

Fig. 7. A fruit-body in median longitudinal section that is a very little older than the one shown in Fig. 6. The pileus fundament is a little larger and the hyphae in the peripheral zone are growing straight outward over its entire surface. ×50.

Fig. 8. Median longitudinal section. By epinastic growth the outer, lateral hyphae have grown downward to form the pileus margin, which appears as a small, deeper stained area on either side. ×30.

Fig. 9. An enlargement of the right side of Fig. 8. Near the center can be seen the pileus margin, whose hyphae extend in a downward and slightly outward direction. Immediately below it the ground tissue is beginning to break away to form the gill cavity. On the outside, at the right, is the loose universal veil. ×115.

Fig. 10. An older stage; the hymenophore primordium appears on either side, on the margin of the pileus. Over the pileus the brown, enlarged hyphae of the blematogen stand straight outward. ×30.

Fig. 11. An enlargement of the right side of Fig. 10, showing details of above mentioned structures and early indication of formation of the gill cavity. ×115.

Fig. 12. Tangential section, showing the uneven condition of the hymenophore. Below the ragged surface of the latter the ground tissue is beginning to break away to form the gill cavity. ×50.

Fig. 13. Tangential section of the same fruit-body, but nearer to the stem. The hymenophore is becoming more even and the gill cavity is much better formed. ×50.

Fig. 14. Median longitudinal section. On the left may be seen the duplex nature of the blematogen over the pileus margin. ×30.

FIG. 15. Left side of preceding figure, more highly magnified. On the outside are the large hyphae of the outer blematogen layer, with an outward and upward direction. Within is the inner blematogen layer, composed of slender parallel hyphae, interlacing with the surface of the pileus above its margin. Below the hymenophore is the gill cavity. ×115.

FIG. 16. Right side of median longitudinal section of a fruit-body a little older than the preceding one. The palisade zone is differentiated and the gill cavity is completed. Extending up over the edge of the pileus margin may be seen hyphae of the inner part of the blematogen. ×115.

FIG. 17. The right side of median section, showing the palisade layer and the universal veil, with the loose area between the latter and the pileus surface. ×25.

FIG. 18. Median section of fruit-body after the gills are formed. The pileus and stem have become very compact through branching and growth of their hyphae. The pileus margin is strongly inrolled, as in all the fruit-bodies sectioned at this stage. The cells of the blematogen hyphae are collapsed and shrunken and show signs of aggregation into the tufts that later become the erect squamules on the surface of pileus and stem. The narrow area of loose tissue between blematogen and pileus surface may be seen here. ×15.

FIG. 19. A fruit-body a little younger than the preceding, showing well the radiating hyphae of the blematogen. ×15.

PLATE XXIX

FIG. 20. The section represented by this photograph is from a fruit-body at a stage after the gills are well formed. The margin of the pileus is strongly incurved and the edge is free from the veil. ×30.

FIG. 21. This section shows well the structure of the cortina. It is composed of the slender hyphae of the inner part of the blematogen and the marginal veil. On the outside are remnants of the outer layer of the blematogen. Since the pileus margin is not strongly inrolled in this particular fruit-body, the edge has not become free from the veil. ×30.

FIG. 22. A portion of the right side of a section showing the pileus with its edge entirely free from the cortina, due to its inrolled character. ×30.

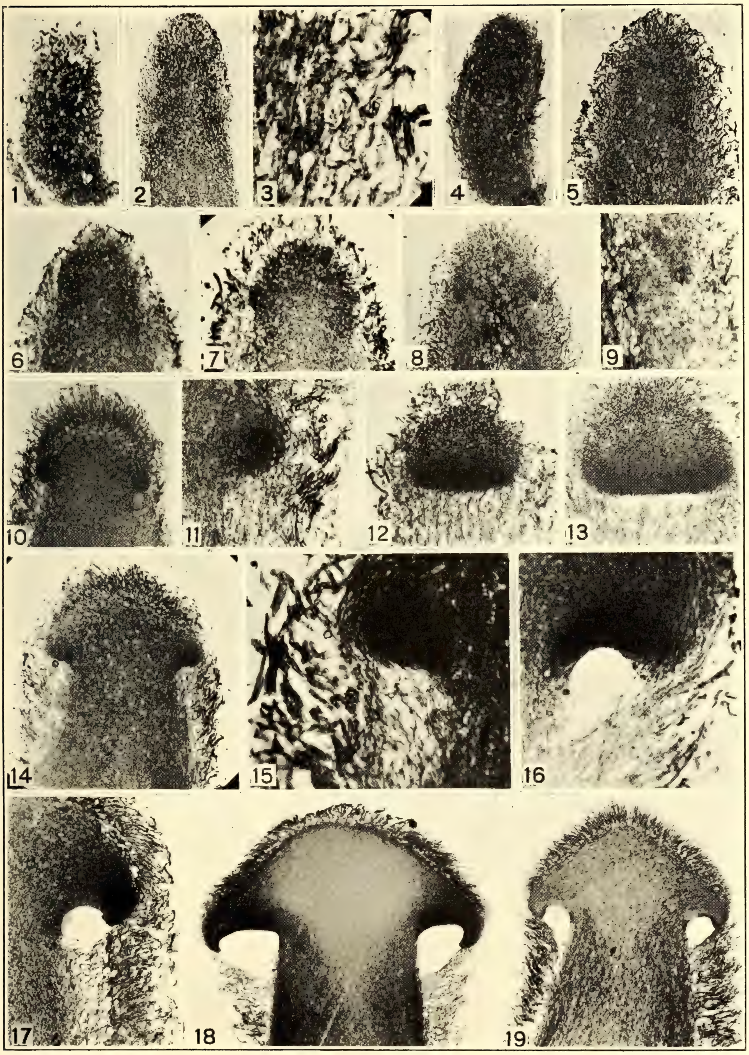
FIGS. 23–27. A series of sections showing the origin and development of the lamellae. In Fig. 23 a section near the pileus margin is shown. In the center is the well developed gill cavity. Above it is the even palisade area of the hymenophore, bordered on either side by primordial tissue. Below is loose ground tissue on the stem. In Fig. 24 the first gill salients are seen as two slightly downwardly projecting broad folds in the palisade. Fig. 25 shows these two salients better developed, toward the stem, and at the right a third is appearing. Fig. 26 represents a section of the same fruit-body tangent to the stem. On the left is the level palisade and on the right development has proceeded further, so that a gill salient has been formed. Fig. 27 is of a section nearer to the center of the fruit-body, showing the same features as in the preceding section. ×112.

FIGS. 28–32. Stages in the origin and development of a dichotomous gill through the branching of a gill salient. The section shown in Fig. 28 is near the stem, and the following figures are nearer the pileus margin in order. ×50.

FIG. 33. A section tangent to the stem at its junction with the pileus, showing two adnate gills. ×30.

FIG. 34. Structure of the mature gills. The hyphae of the trama turn outward on the sides and contribute to the formation of the hymenium, which shows as the deeply stained layer covering the gill. ×30.

FIGS. 35–37. Tangential sections in the inrolled margin of a nearly mature fruit-body. In Fig. 35 the section is tangent to the "backs" of the gills. In Fig. 36 the lamellae appear to grow across the gill cavity as bars, with a separate cavity between each two gills. In reality it is the backs of the gills seen in section, and their attachment below, as well as above, represents their point of origin. Due to the inrolled pileus margin the tissue at either end of the "bars" belongs to the same morphological under surface of the pileus. Fig. 37 shows the origin of secondary gills between the primary lamellae, both above and below. ×15.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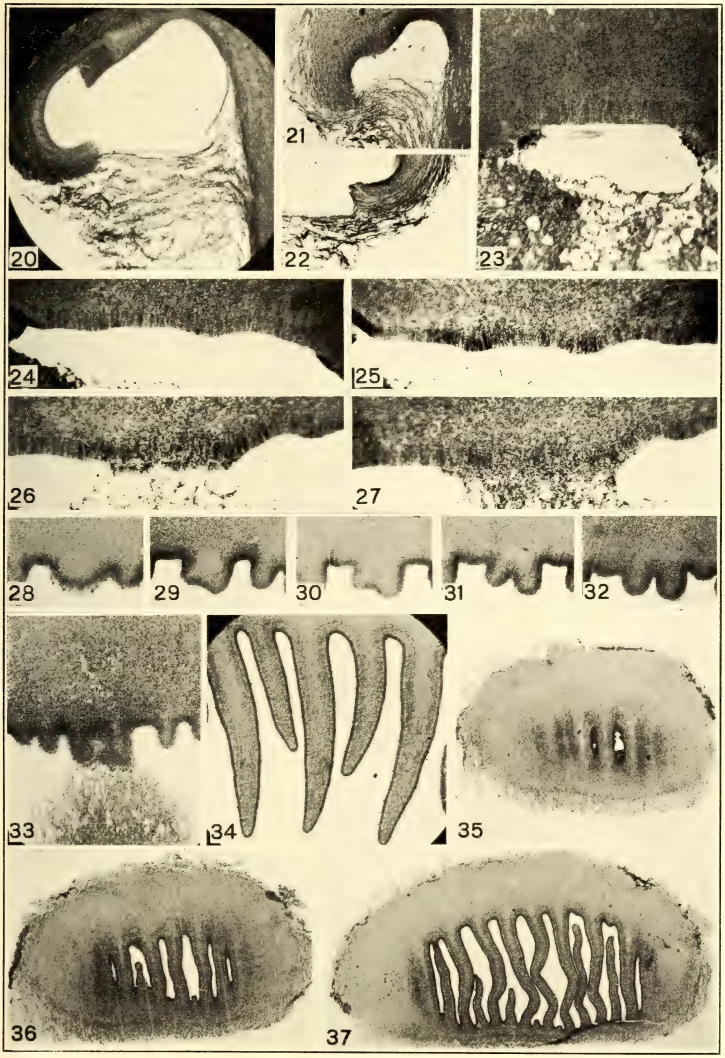
SAWYER: DEVELOPMENT OF CORTINARIUS PHOLIDEUS.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SAWYER: DEVELOPMENT OF CORTINARIUS PHOLIDEUS.

LEAF-STRUCTURE AS RELATED TO ENVIRONMENT

Herbert C. Hanson

Introduction

This investigation was begun during the summer of 1915 and carried on for about a year. Preliminary observation showed that the leaves growing in the sun at the south periphery were thicker than the leaves growing in the shade at the center of the same tree. The purpose of the investigation was to find out the exact differences in the structure of the leaves from the two positions, and to compare by measurements of the factors the environments in which the leaves were growing. The factors measured were light, evaporating power of the air, temperature, humidity and wind. A study of transpiration was also made during the summer of 1916.

Historical

The relation of leaf structure to factors of the environment has been studied by numerous investigators. Although very few works give factor measurements, some of the more recent investigations on leaf structure are here reviewed. One of the most detailed works is by Mrs. Clements (12), which shows the differences in the structure of numerous hydrophytic, mesophytic and xerophytic leaves, and of sun and shade leaves on different plants of the same species. The physical factors, light, water-content of the soil, humidity and temperature, were measured for most of the habitats in which the plants studied were growing. Good reviews of earlier articles on leaf structure, and of explanations concerning the structure and formation of palisade cells, are given by Mrs. Clements.

Experiments performed by Eberhardt (14) showed that humid air caused an increase in the size of the leaf, in the amount of chlorophyll and in root development; while dry air caused an increase in the thickness of the cuticle, in the number of stomata, and in the amount of sclerenchyma, woody tissue and palisade.

Brenner's (9) experiments on various succulent plants are interesting and important. Plants grown in moist air showed the following changes as compared with plants growing in the normal environment: storage tissue, the fibro-vascular bundles, air-spaces, dry weight, ash and acid content of the leaves all decrease; chlorophyll tissue increases; chlorophyll cells become more isodiametric; walls of epidermal cells become wavy; number of stomata per leaf increases, although the number for a unit area may be the same; and epinasty replaces hyponasty, so that the leaves grow at right angles to the stem. He believed the air factors, not the soil factors, determined these changes, but that physical explanations do not always seem adequate to account for the changes.

Brenner (10) concludes from his anatomical and experimental study on Quercus leaves that modifications caused by the environment are hereditary and may develop into new species.

Bonnier (7) selected about fifty species of perennial plants at Fontainebleau. Each plant experimented upon was split, one part planted at Fontainebleau, the other at Toulon. The plants set out at Toulon became like the wild plants surrounding Toulon in leaf and and wood structure and in external characters.

Hansgirg (18) gives descriptions in detail of more than fifty types of leaves, with a discussion of their ecological advantages.

Chrysler (11) compared the leaf structure of nine strand plants from the Atlantic coast near Wood's Hole, Massachusetts, and from the vicinity of Chicago. The leaves of the maritime plants were from less than once to a trifle over twice as thick as the inland plants. The increase in thickness was mostly due to increased palisade development. Greater compactness in tissue and increased thickness of the outer epidermal wall were found in certain plants. The amount of salt in the soil is given as the probable cause for the variations.

Boodle (8) in his experimental study on the leaves of *Pteris aquilina* Linn. found that leaves grown in dry and exposed situations had a xerophytic structure while those grown in sheltered positions were more mesophytic. The former leaves possessed hypoderm, the latter had none and the palisade was poorly developed, or entirely missing. The same differences were found on leaves of the same plant, or on different parts of the same leaf. A plant that had been producing shade leaves in a moist greenhouse, produced sun leaves when placed in a garden. The mature structure of the leaf is not determined at an early stage in the leaf's growth.

Copeland (13) found great variations in the shape and thickness of leaves on the same branches of various plants.

Hesselman (23) brought out many important facts in his very detailed and quantitative work. Leaves formed in the stronger light of the forest in the spring developed more palisade than leaves that developed later in the weaker light. The trees that had high light requirements, Betula, Fraxinus, produced sun-leaves, while the trees of low light requirements, Quercus, Corylus, produced both sun and shade leaves. The trees in the first group produced starch in all the leaves, while those in the latter group produced no starch in the innermost leaves. Shade leaves make more starch than sun leaves of the same species when the light is equal. The production of starch decreases from spring to summer in the forest more in sun plants than in shade plants. The respiration of sun plants is far greater than of shade plants. If the leaf surface is equal, transpiration increases with the amount of palisade. Sun plants in the sun transpire much more than shade plants in the shade. The work also contains good representations of ecological structures of plants.

Bergen (4) compared the transpiration, color, size and the general structure of sun and shade leaves of the same individuals of the following: *Olea europea sativa, Pistacia, Lentiscus, Quercus, Ilex, Rhamnus Alaternus*. He found the ratio in thickness of sun leaves to shade leaves to be 1.5–3.7 to 1. The sun leaves had thicker cuticle, more palisade, smaller air spaces, greater bundle development, 15 percent more stomata as determined by two observations, greater scaliness, darker color and smaller area. The greater transpiration in the sun leaves was due to their greater activity, because their larger stems and bundles transfer the water more quickly, and because the greater thickness of the leaves afforded a larger interior evaporating surface. In another article (5) Bergen compares the thickness and transpiration of new and old leaves. From the result of another study (6) he states that "it is undoubtedly a fact that the great majority of woody dicotyledons have leaves which when freely exposed to the sun are concave on the upper surface and that this concavity usually lessens or disappears in the case of much shaded leaves on the same plant."

Oltmans (26) noted that the leaflets growing on the south periphery of *Robinia Pseudo-Acacia* trees were concave, while those on the north periphery were flat. Wiesner (32) also observed that while the upper surface of peripheral leaves was concave, the leaves in the shade of the same tree were usually flat.

Herriott (22) gives the frequency and violence of the wind and the peaty soil as causes for the xerophytic leaf structure of some New Zealand plants.

Raunkiaer (27) found that palisade tissue was equally well developed in the leaves of certain plants above the water and to a depth of twenty centimeters. Below this the length of the palisade cells gradually decreases. No palisade tissue was distinguished in shade plants under water, nor above, to a height of about thirty centimeters. His discussion of the causes of palisade development and the orientation of the palisade cells is valuable.

Lubimenko (25) determined that the chloroplasts of certain ombrophilous plants, as Tilia and Abies, were greater in size and in sensitivity than the chloroplasts of ombrophobous plants, as Pinus and Betula. The pigment was more concentrated in the former group.

Baumert's (3) work contains a good review on the literature of structures protecting leaves from light. From his own experiments he found that a thick white coating of hairs as in *Centaurea candidissima* reduced the heat in the leaf 37.5 percent, shininess reduces the heat 30 percent, and a wax coating up to 13.6 percent. Wiegand's (31) experiments show the efficiency of hairy and cutinized coverings in reducing the water loss by transpiration.

Areschoug (2) maintains that well-developed, compact palisade tissue reduces transpiration, despite Hesselman's experiments to the contrary.

Ewart (15) experimenting upon *Tilia europaea* found that mature leaves do not increase in size when most of the leaves are removed from the tree. The increased size of the new leaves which replace those defoliated is due to the increase in the number of cells.

Sampson and Allen (29) found that the sun leaf transpired from two to four times as much as the shade leaf of the same species whether the leaves were placed in the sun or shade. This was explained by the greater number (20 percent–60 percent) of stomata in the sun leaf.

Harshberger (20, 19) investigated the leaf structure of strand plants in New Jersey, and sand dune plants in Bermuda. He states that the xerophytic structures are due to intense light, strong winds, and in rare cases to salt spray. The unequal illumination of the two sides of the leaf causes the formation of palisade and sponge tissues.

Renner (28), in discussing the relation of wind to transpiration, says that the transpiration of small mature leaves is increased to a far greater extent by the wind than that of large leaves. This is explained by the vapor cap which is thicker about the larger leaves. If the air were absolutely quiet Renner believed the thickness of the vapor cap would vary with the diameter of the leaves.

Adamson (1) found the xerophytic structures in leaves of certain species of Veronica to consist in reduced leaf surfaces, reduction in intercellular spaces, and an increase in the thickness of the cuticle.

Livingston and Brown (24) in their study of the daily march of transpiration showed that the water content of leaves falls during the day and rises during the night.

Starr (30) compared the structures of stems and leaves of plants on dunes and on flood plains. She discusses the ecological factors of the dunes, but no measurements are given. The leaves of the dune plants owe their greater thickness to increased palisade tissue chiefly.

Ganong (16) states that one of his students found that the petioles from the exposed part of a tree were larger than those from more sheltered positions.

Haberlandt (17) says that a comparison of the vigorously transpiring sun leaves and the feebly transpiring shade leaves of the same plant shows an increase in the linear dimensions of the vascular system in the sun leaves.

Hasselbring (21), working on tobacco plants growing in the sun and in the shade, showed that the proportions of dry matter, and the production of plant substance for equal areas of leaf surface were greater in the sun plants. The shade plants transpired 186.99 cc. of water in producing one gram of plant substance, while the sun plants transpired 241.72 cc. He found the water content of leaves from sun plants to be 81.39 percent and of leaves from shade plants to be 83.68 percent. The sun plants transpired .412 cc. while the shade plants transpired .224 cc. per square decimeter of leaf surface per hour.

METHODS

The readings of the environmental factors were made in the sun among the leaves on the south periphery of untrimmed isolated trees and at the apex of trees growing in the forest. At a height corresponding to the sun readings on isolated trees, readings were taken in representative positions within the crowns; and for the forest trees readings were taken among the lowest leaves. For a given species the readings were taken upon the same individual in the forest or in isolated positions. Care was taken so that the various factors were measured in the same positions in the crown or at the south periphery of each tree. Cytological material was collected from leaves growing in these positions respectively. Pieces from about twenty leaves from each position were killed in chromo-acetic acid and in Juel's killing solutions. The material from Juel's solution gave the best specimens for study. The ordinary paraffine method was used in preparing the material.

The response of the leaves to the environmental factors was shown in transpiration; in the green and dry weights and water content of given leaf areas; in the thickness of the leaf and its parts, palisade, sponge, upper and lower epidermis and the cuticle; in the compactness of the tissues; in the structure of individual cells; and in the macroscopic characters, as area and lobing.

The Physical Factors

1. *Light*

The light was measured by means of the Clements' photometer between 11 A.M. and 2 P.M. in August, 1915. Four readings were taken within the crown of isolated trees and among the lowest leaves of the forest trees for four or five individuals of ten species. From these 16 to 20 readings for each species the light values, as arranged in the following table, were averaged.

Table I

Showing the Light Values in the Crown of Isolated Trees and at the Base of Forest Trees

Species	Position of Trees*	Light Value in Crown or at Base
Acer saccharum Marsh.	Isolated	0.0175—crown
	Forest	0.0076—base
Tilia americana L.	Isolated	0.0688—crown
	Isolated (L.)	0.1000—crown
	Forest	0.0086—base
Quercus macrocarpa Michx.	Isolated	0.1132—crown
	Forest	0.0754—base
Quercus rubra L.	Forest	0.0425—base
Quercus alba L.	Forest	0.0100—base
Acer saccharinum L.	Isolated	0.0406—crown
	Isolated (L.)	0.0380—crown
Acer negundo L.	Isolated	0.0979—crown
Ulmus americana L.	Isolated	0.0770—crown
Fraxinus pennsylvanica Marsh.	Isolated	0.0497—crown
Celtis occidentalis L.	Isolated (L.)	0.0591—crown

* Trees marked (L.) in Lincoln, all others in Minneapolis.

The order of the light values in isolated trees, beginning with the lowest, is seen to be *Acer saccharum, Acer saccharinum, Fraxinus pennsylvanica, Celtis occidentalis, Tilia americana, Ulmus americana, Acer negundo,* and *Quercus macrocarpa.* In the forest the order is *Acer saccharum, Tilia americana, Quercus alba, Quercus rubra,* and *Quercus macrocarpa.* Comparing the isolated and forest individuals of the same species it may be noted that the trees in the forest have the lower light intensity.

2. *Evaporating Power of the Air*

Livingston's standardized porous cups were used for measuring the evaporating power of the air. The cups were placed in the trees between 8 and 10 A.M. and taken down about 5 P.M. The ratios of the amounts of water evaporated from the crown to that from the south periphery in isolated trees, or from the base to the apex of the crowns of forest trees were lowest in isolated trees of *Ulmus americana* and *Acer negundo* (1 : 1.44, 1 : 1.48) and greatest in isolated *Acer saccharinum* and *Tilia americana* (1:2.3, 1:2.2). The ratios of five other species ranged between these. The ratios in the isolated trees were always less than the ratios in forest trees of the same species, for example, in the isolated tree of *Quercus macrocarpa* the ratio was 1 : 1.3, while in the forest tree it was 1:2.2. This is due to the greater humidity at the base of the forest tree as compared with the humidity within the crown of the isolated tree.

The temperature and humidity data indicate that the differences in the amounts evaporated in the sun and in the shade positions cannot be explained by these two factors alone. The movement of the air seems to be the controlling factor. As the water evaporates from the cups in the exposed situations it is rapidly carried away from the vicinity of the cups by air currents. As the wind data show, there is much less air movement within the crown than at the south periphery, so it is very probable that a vapor blanket is formed about the cups within the crown. This blanket would very likely be formed also about each leaf. Renner (28) concluded that the thickness of the vapor blanket varies with the leaf size. In this way the saturation deficit would be decreased, and evaporation and transpiration lowered. Radiant energy may also play an important rôle in causing the differences, because it is greater in the exposed than in the sheltered positions. As the readings were not taken upon all of the trees at the same time, comparisons between species cannot be made except in a very general way.

3. *Temperature*

Numerous simultaneous readings of temperature were made in the shade within the crown and in the sun at the south periphery upon several isolated trees of *Acer saccharum*, *A. saccharinum*, *Tilia americana* and *Fraxinus pennsylvanica*. The greatest differences between the two positions were 2.8° C. at 2:50 P.M. on *Acer saccharum* and 1.8° C. at 1:00 P.M. on *Tilia americana*. Usually the difference was about 1° C. This difference was caused by the stronger light at the south periphery.

4. *Humidity*

Humidity readings were made with cog psychrometers simultaneously within the crown and at the south periphery of the same trees that were used for temperature readings. The greatest differences were found on *Acer saccharum*, 16 percent, at noon. The greatest difference in the case of *Tilia americana* was 9 percent at 2 P.M. The usual differences in the humidity of the two positions was from 1 percent to 2 percent. The greatest factor in causing these differences was the movement of the air. Dense crowns impeded the free movement of the air far more than open crowns, so that trees with dense crowns, as *Acer saccharum*, showed greater differences than trees of open crowns. The greatest saturation deficits were always found on the exposed parts of trees from 1 P.M. to 3 P.M.

5. *Wind*

Numerous readings of wind velocity were taken by hand anemometers, operated simultaneously in the two positions. The ratios of the velocity within the crown to that at the periphery have been determined from several readings on each tree. In *Acer saccharum* the ratio was 1:2.2; in *A. saccharinum*, 1:2.1; in *Quercus macrocarpa*, 1:2.0; in *Ulmus americana*, 1:1.6; in *Tilia americana*, 1:1.4; in *Fraxinus pennsylvanica*, 1:1.4; in *Acer negundo*, 1:1.3.

The amount of air movement within the crown depends upon the openness of the crown. The two extremes in crown density in the above series were *Acer saccharum* and *Acer negundo*. As seen from the figures the least air movement within the crown in comparison to that at the south periphery was found in the former, and the greatest in the latter. When the wind velocity was high or if it came in gusts there was less difference in the air movement within the crown and at the south periphery than when the velocity was low. This is explained by the fact that the leaves and branches were more effective in keeping the wind out of the crown when the wind velocity was low than when it was high. As the wind velocity was not the same when all the readings were made, the ratios of the various trees cannot be directly compared with each other. It is highly probable that if readings were taken on *Acer negundo* under the same velocity as on *Acer saccharum*, *i. e.*, 68.9 meters per minute instead of 58.6, the ratio of wind in the crown to that of the south periphery would be less than 1 : 1.3. In the case of *Acer saccharum* as high a ratio of crown to south periphery as 1 : 8.1 was found when the wind velocity was regular and fairly low.

Readings were taken with two cup anemometers on a *Fraxinus pennsylvanica* near Lincoln. The readings were made every half hour from 9:45 A.M. to 3:45 P.M. As the wind came from the northwest, readings were made at the northwest periphery and within the crown. The wind velocity within the crown was found to vary from 32 percent to 52 percent of that at the northwest periphery. At 9:45 A.M. the velocity within the crown was 3.7 kilometers per hour and at the northwest periphery 11.6. At 3:45 P.M. the velocity within the crown was 6.6 km., and at the periphery 12.7 km.

EFFECTS OF THE PHYSICAL FACTORS

1. *Transpiration*

Method

Branches from the center of the crown and from the south periphery, bearing numerous leaves, were cut under water. These branches were securely fastened by rubber tubing to the base of burette tubes graduated to tenths of cubic centimeters. The amount of water in each tube was about equal throughout the experiment, so that there would be no error due to unequal heads of pressure. The tubes, with the branches attached so as to be upright, were fastened in representative situations in the center or at the south periphery. Readings were usually taken simultaneously in both situations every 15 minutes. The leaf area was measured from prints on Kresko paper by means of the planimeter.

Results

On July 11, 1916, four sets of branches were arranged on a *Fraxinus pennsylvanica* growing isolated in a pasture. The tree had a well-shaped, fairly dense crown. One potometer containing south periphery leaves and one containing center leaves were placed among the leaves at the south periphery about 10 feet above the ground. Two more potometers, one containing south periphery leaves, the other center leaves, were placed within the crown at about the same height as the other two potometers. There was very little wind and the sky was very hazy. The transpirations for periods of 15 minutes and for a total of three hours are given in the following table:

TABLE 2

Transpiration of Fraxinus Pennsylvanica on July 11, 1916

	Time P.M.	Total Loss in Cc.	Loss per Sq. Dcm. in Cc.		Time P.M.	Total Loss in Cc.	Loss per Sq. Dcm. in Cc.
Leaves from south periphery placed at south periphery	1:08			Leaves from center of crown placed at south periphery	1:23		
	1:23	0.5	0.0212		1:38	0.10	0.0054
	1:38	0.9	.0383		1:53	.20	.0110
	1:53	1.2	.0512		2:08	.20	.0110
	2:08	1.3	.0554		2:23	.35	.0194
	2:23	1.45	.0617		2:38	.35	.0194
	2:38	1.45	.0617		2:53	.35	.0194
	2:53	1.4	.0596		3:08	.30	.0166
	3:08	1.45	.0617		3:23	.35	.0194
	3:23	1.45	.0617		3:38	.55	.0304
	3:38	1.35	.0574		3:53	.45	.0248
	3:53	1.3	.0554		4.08	.35	.0194
	4:08	1.15	.0488				
Total	3 hrs	14.90	.634		2 ¾ hr	3.55	0.1962
Leaves from south periphery placed at center of crown	1.08			Leaves from center of crown placed at center			
	1:23	.45	0.0175		1:23		
	1:38	.55	.0216		1:38	0.20	0.0087
	1:53	.65	.0254		1:53	.25	.0110
	2:08	.75	.0293		2:08	.25	.0110
	2:23	.80	.0313		2:23	.35	.0155
	2:38	.90	.0352		2:38	.30	.0132
	2:53	.95	.0372		2:53	.40	.0177
	3:08	1.00	.0392		3.08	.40	.0177
	3:23	1.05	.0411		3:23	.40	.0177
	3:38	1.10	.0431		3:38	.40	.0177
	3:53	0.90	.0352		3:53	.35	.0155
	4:08	0.90	.0352		4:08	.35	.0155
Total	3 hrs	10.00	.3913		2¾ hr	3.65	.1612

The losses of the south periphery leaves were from 3 to 6 times as great as those from the center leaves when placed in their native situations. It is interesting to note that the south periphery branch in the center of the crown lost more water than the center branch at the south periphery. This may be due to several causes; probably the stomata in the leaves of the center branch closed when exposed to the sun (but other experiments indicate that this was not the case), or the center leaf may have been transpiring up to its full capacity. The south periphery leaf had greater capacity for transpiration than the center leaf, because of its greater amount of solid matter and chloroplasts. Graphs for the two potometers of center leaves give evidence by their parallelism for this view, indicating that the center leaves placed in the center are transpiring at almost full capacity. Sampson and Allen (29) account for the greater transpiration of the sun leaves, because they have from 20 to 60 percent more stomata. Hesselman (23) accounts for the increase in transpiration in his statement that the leaf surface being equal, plants transpire more as they have greater development of palisade. Bergen (4) explains the greater transpiration in the sun leaves by their greater activity, by their greater thickness affording a larger interior evaporating surface, and by their larger bundles and stems which would transfer the water more quickly. These graphs also show by their abrupt changes that the center leaves are more responsive to the environmental factors than the south periphery leaves. This is explained by the greater amount of protective material, as thicker cuticle, greater thickness of the leaf and more solid material in the south periphery leaves.

On July 22, 1916, a clear, hot day, with a light south breeze, three sets of branches were used in an experiment on an isolated *Ulmus fulva* Mich. Each set contained one potometer of a south periphery branch placed at the south periphery, and one potometer of a center branch placed at the center. The sets were run from 1¼ hours to 2¼ hours, readings being taken every 15 minutes. The greatest differences in the water loss between the south periphery and center leaves occurred in the first set where in 1½ hours the south periphery leaves lost 1.956 cc. per square decimeter, while the center leaves lost 0.1614 cc. per square decimeter, a ratio of about 12 : 1. The least differences were in the third set, where the south periphery leaves in 2¼ hours lost 3.10 cc. per square decimeter, while the center leaves lost 0.638 cc.

per square decimeter, a ratio of about 5: 1. As the readings for the third set were made in the morning and those of the first set in the afternoon, the difference in the ratios of the two sets are most likely due to the lower humidity, higher temperature and stronger light, causing greater differences between the center and south periphery during the first experiment.

The differences in the transpiration of *Ulmus fulva* leaves is about twice as great as the differences in *Fraxinus pennsylvanica* leaves of July 11. This greater ratio between the exposed and sheltered leaves in Ulmus is partly due to weather conditions. On July 11 the temperature was lower, the humidity higher, and the sunlight was less bright than on July 22. The physical factors in the center and at the south periphery were therefore more alike.

On June 23, 1916, a cloudless, warm day with a light breeze, an experiment was performed on a well-formed isolated *Acer saccharinum*. Two potometers of south periphery leaves were prepared, one was placed at the south periphery, the other at the center. A potometer of center leaves, also, was placed at the south periphery. At the end of 50 minutes readings were made and the positions of all potometers were changed from south periphery to center or vice versa, and allowed to run 50 minutes after about 5 minutes for adjustment had been allowed.

The potometers, containing south periphery leaves, placed at the south periphery, lost 10.5 cc. and 11.95 cc. When these were moved to the center of the tree the losses were 3.95 cc. and 2.85 cc., respectively. The center leaves lost 5.4 cc. at the south periphery and 1.2 cc. in the center. The temperature at the periphery of the tree was practically the same during the 105 minutes. The evaporation from Livingston's porous cups was 4.6 cc. in 50 minutes at the south periphery and 3.4 cc. in the center.

The amount of water lost by transpiration is increased from about 3 to over 4 times when the potometer is changed from center to the south periphery. The small differences in temperature and evaporation in the two positions compared to the great differences in transpiration show that plants, compared with mechanical apparatus, are more sensitive to environmental factors. Comparison cannot be made between the three potometers in this experiment as the leaf area was not measured. Comparison can be made only between the positions of the same potometer.

2. *Surface Area and Lobing*

In nearly all cases the leaves from the center or from the base of the crown were larger than those from the south periphery or apex. The greatest differences were found in the second crop of leaves during the season on forest forms of *Tilia americana,* in the first crop leaves of isolated *Fraxinus pennsylvanica* and in the first and second crops of leaves of isolated and forest individuals of *Quercus macrocarpa.* The production of a second crop of leaves was due to the warm weather in June following a cold spring. The least differences in surface extent were found in the isolated *Tilia americana* and *Acer saccharum.* Usually the leaves from the exposed positions were more deeply and narrowly lobed, and more prominently toothed than the leaves from the protected positions. The lobing of the south periphery and the apex leaves of *Acer saccharum* was less deep than in the center or base leaves.

3. *Green and Dry Weights: Water Content*

Two methods were employed in determining the green and dry weights and the areas of the leaves weighed; one by means of the Ganong leaf area cutter, the other by weighing entire leaves and then determining their area by means of the proportional weights, using solio paper for the leaf prints. Sufficient material was used in each method so as to render the error negligible. The leaves were always collected late in the afternoon. The green and dry weights per square decimeter of leaf surface and the water content are given in the following table:

In every case the green and dry weights were lower, and the water content higher, in the shaded than in the sunny positions. The ratio between the weights is greater in the dry weights than in the green weights, showing that more solid material was laid down in the leaves where the light and other factors were more intense. The highest water content was found in the shade leaves of *Acer saccharum, A. saccharinum* and *Fraxinus,* while the lowest was in *Quercus alba* and *Q. rubra.* The greatest differences in water content between the sun and shade leaves were found in *Fraxinus* and *Acer saccharum,* while the smallest occurred in *Quercus rubra* and *Q. alba.* According to the amount of dry material in the leaves at the south periphery or apex the trees fall into the following order: *Fraxinus* 1.639 g., *Q. macrocarpa* 1.272 g., *Q. rubra* 1.190 g., *Q. alba* 1.173 g., *Tilia americana*

TABLE 3

Green Weight, Dry Weight and Water Content of Leaves from the Center and South Periphery of Isolated Trees, and from the Base and Apex of Forest Trees

	Position of Tree	Green Weight		Dry Weight		Water Content		
		Grams per Sq. Dcm.	%	Grams per Sq. Dcm.	%	Grams per Sq. Dcm.	% of Green Wt.	% of Dry Wt.
Acer saccharum (isolated)....	Center..	0.835	46	0.354	38	0.481	74.6	135.9
	So. Per..	1.760	100	0.937	100	0.823	52.1	87.9
Acer saccharum (forest).....	Base....	0.878	47	0.361	35	0.517	58.8	143.2
	Apex...	1.882	100	1.029	100	0.853	45.4	82.9
Tilia americana (isolated)...	Center..	1.078	58	0.038	51	0.698	64.8	183.7
	So. Per..	1.861	100	0.745	100	1.116	59.9	149.8
* *Tilia americana* (forest)....	Base....	0.745	29	0.223	20	0.522	70.1	234.1
	Apex...	2.589	100	1.114	100	1.475	56.9	132.4
Quercus alba (forest)........	Base....	1.354	74	0.817	70	0.537	39.7	65.7
	Apex...	1.825	100	1.173	100	0.652	35.7	55.6
* *Quercus alba* (forest)......	Base....	1.276	58	0.467	47	0.809	63.4	173.2
	Apex...	2.186	100	0.997	100	1.189	54.4	119.2
Quercus rubra (forest).......	Base....	1.458	63	0.699	58	0.759	52.1	108.5
	Apex...	2.331	100	1.190	100	1.141	48.9	95.9
Quercus macrocarpa (isolated).............	Center..	1.469	66	0.579	46	0.890	60.7	153.8
	So. Per..	2.227	100	1.272	100	0.955	42.9	75.1
Ulmus americana (isolated)..	Center..	0.906	71	0.309	55	0.597	65.9	193.2
	So. Per..	1.274	100	0.560	100	0.714	56.0	127.5
Fraxinus pennsylvanica (isolated)............	Center..	1.428	59	0.467	29	0.961	67.3	205.8
	So. Per..	2.440	100	1.639	100	0.801	32.8	48.8
Acer saccharinum (isolated)..	Center..	0.723	60	0.229	42	0.494	68.3	215.9
	So. Per..	1.211	100	0.546	100	0.665	54.9	121.8

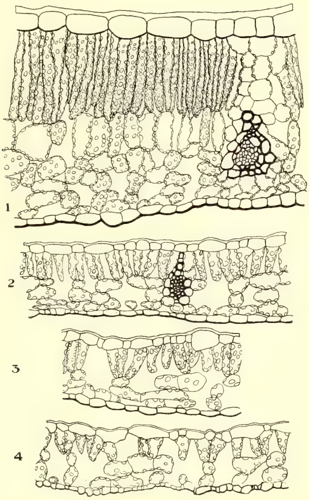
* Second growth leaves of the season.

1.114 g., *A. saccharum* 1.029 g., *Ulmus americana* 0.560 g., *A. saccharinum* 0.546 g. The relation of this sequence to tolerance is noteworthy. The green weights of the leaves from the same position do not show so much relationship, but *Fraxinus*, *Q. rubra* and *Q. macrocarpa* weighed most, while *Ulmus* and *A. saccharinum* weighed least.

Two crops of leaves were produced by many trees during the growing season of 1915. The first crop appeared under cold and humid conditions. The second crop developed about June 25 when the warmer and drier summer weather had arrived. In the latter part of August the first crop of leaves showed a much lower water content than the second crop. The total green weight of the base leaves of the second crop was less and of the apex leaves greater than that of the first crop. The explanation of this probably is that the new base leaves were developed under lower light intensity caused by the shade of the first leaves; while the new apex leaves appeared when the light intensity and the evaporating power of the air were greater than when the first crop developed. In the former case less solid material, and in the latter, more solid material would form in the leaf cells.

4. *Comparison of Leaf Structure*

The detailed microscopical study and measurements of the leaf structure of various trees are summarized in the following observations for each species. This detailed study was made upon cross sections of from five to ten representative leaves from each position of the trees.



All figures are made from photographs of camera lucida drawings of sections of leaves. The leaves were taken from the various positions indicated below. Except as indicated, all material was from the vicinity of Minneapolis, Minn.

Figs. 1–4. *Acer saccharum.* Fig. 1, Isolated tree. Leaves from south periphery. Fig. 2, Isolated tree. Leaves from center of crown. Fig. 3, Forest tree. Leaves from base. Fig. 4, Forest, seven-year-old tree. Leaves from base.

The trees were selected carefully so as to secure specimens typical of the species. Permanent mounts of these sections were made by the paraffin process. Care was taken so that the sections were cut from the same part of leaves of like age and that the measurements were made in typical parts of the sections so that no error would be caused by the thickening due to fibro-vascular bundles.

Acer saccharum.—The study of an isolated tree showed that the center leaves were on an average 38 percent as thick as the south periphery leaves. This increase was caused mostly by the great palisade development, the thickness of the palisade tissue in the center leaves being about 25 percent that of the south periphery leaves. In the center leaves the palisade made up 38 percent of the total thickness, while in the south periphery leaves it made up 58 percent. The thickness of the sponge tissue and upper epidermis was about one half as great, and the lower epidermis three fourths as great in the center leaves as in the south periphery leaves.

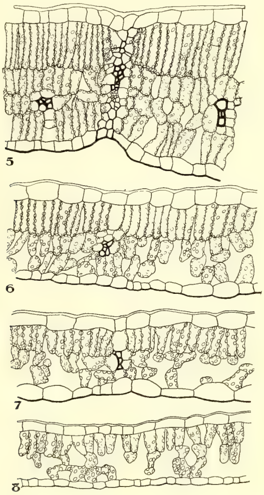
Great differences in structure were found in the leaves from the two positions. The south periphery leaves had two layers of palisade and these layers were far more compact than the single layer in the center leaves. The sponge tissue was more compact, the bundles and water storage tissue more abundant, the cells in the upper and lower epidermis more regular, and the number of crystals greater in the south periphery leaves than in the center leaves.

The weight of the green leaves per given area and the weight of the water-free leaves in the center were 46 percent and 38 percent of the weights at the south periphery. The water content based on green weight of the center leaves was 75 percent, and of the south periphery leaves 52 percent.

Factor measurements showed that the amount of evaporation and the rate of the wind in the center of the tree were respectively 67 percent and 28 percent of the amounts at the south periphery. The light within the crown was 0.0086, while at the south periphery it was 1.00.

Less pronounced differences were found in the leaf-structure of trees growing in the forest. The thickness of the leaves growing at the base of the trees were 44 percent the thickness of the leaves at the apex. The apex leaves were 77 percent the thickness of the south periphery leaves of the isolated tree and the base leaves 92 percent the thickness of the center leaves. The weight of the green leaves and the weight of the water-free leaves of the base leaves were 47 percent and 35 percent the weights of the apex leaves. The light intensity of the base leaves was 0.0076. Leaves were found growing in a light intensity of 0.0024, fifteen centimeters above the ground. The thickness of these leaves was 30 percent the thickness of the south periphery leaves of the isolated tree. The palisade and sponge tissue were very loose.

Tilia americana.—The total thickness of the center leaves of isolated *Tilia americana* in Minneapolis was 52 percent the thickness of the south periphery leaves. This difference was caused chiefly by the great increase of palisade tissue, the center leaves having only 22 percent as much palisade as the south periphery leaves. The palisade tissue composed 34 percent of the thickness of the center leaves, and 81 percent of the other. The sponge tissue is changed to palisade in the south periphery leaves. There are four layers, usually, of palisade in the latter leaves and only one in the former. The cells in the center leaves are larger, more irregular, more often funnel-shaped, the air-spaces are larger and more numerous, the bundles and



Figs. 5–8. *Tilia americana.* Fig. 5, Isolated tree. Leaves from south periphery. Fig. 6, Isolated tree. Leaves from center of crown (Lincoln). Fig. 7, Isolated tree. Leaves from center of crown. Fig. 8, Forest tree. Leaves from base.

water storage cells are more poorly developed, the crystals are less numerous, and the side walls of the epidermal cells are more wavy. The weight of the green leaves from the center was 58 percent the weight of the south periphery leaves, the weight of the water free leaves from the center 51 percent. The water content of the center leaves was 65 percent, of the south periphery leaves 60 percent.

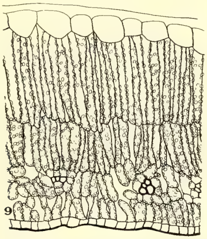
The amount of evaporation and the wind in the center were 77 percent and 65 percent of the amounts at the south periphery. The light intensity in the center was 0.0353.

The center leaves of an isolated tree examined in Lincoln had thicker leaves than the isolated tree in Minneapolis, although the south periphery leaves were thinner. As the light intensity in the center was 0.115, and the evaporation 59 percent that of the south periphery; it seems probable that the increase in light intensity accounts for the increase in the leaf thickness.

As in *Acer saccharum* the leaves on forest individuals of this species are thinner than on isolated individuals. The apex leaves of forest trees are about the same thickness as the south periphery leaves of isolated trees, while the base leaves of the forest trees are 69 percent the thickness of the center leaves of isolated trees. The decrease in thickness may be accounted for by the increased humidity and the lower light intensity, 0.00865, in the forest, as compared with the isolated tree.

The second crop leaves from the base of the forest individuals were thicker (31 percent) than the first crop while the apex leaves were thinner (17 percent). The weight of the green leaves and the weight of the air-dried leaves at the base were 29 percent and 20 percent that of the apex leaves per given area. Although the difference in the thickness of the first and second crop apex leaves was only 17 percent, the structure of the second crop leaves was far more mesophytic as seen in the amount of air space, number of bundles, and water storage cells.

Quercus macrocarpa.—The center leaves of a well-formed, typical isolated tree were 61 percent of the thickness of the south periphery leaves. The increase in thickness was due chiefly to the increased development of palisade tissue. The amount of palisade in the center leaves was 37 percent of that in the south periphery leaves. The amount of sponge in the center leaves was over twice as great as in the south periphery leaves, showing that most of the sponge tissue had become palisade. In the center leaves the palisade made up 46 percent of the total thickness; the sponge, 34 percent; the upper epidermis 13 percent; the lower epidermis 7 percent. In the south periphery leaves the palisade made up 75.8 percent of the total thickness; the sponge 7.8 percent; the upper epidermis 10.6 percent; the lower epidermis 5.8 percent. The weight of the green leaves and the weight of the water-free leaves in the center were 66 percent and 46 percent of those at the south periphery. The water content of the former leaves was 61 percent, of the latter leaves 43 percent.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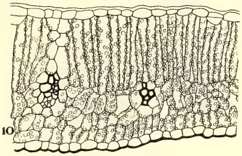
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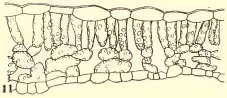
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Fig. 9. *Quercus macrocarpa.* Isolated tree. Second growth leaves from south periphery.

Figs. 10–11. *Acer saccharinum.* Fig. 10, Isolated tree. Leaves from south periphery (Lincoln). Fig. 11, Isolated tree. Leaves from center of crown.

The amount of evaporation and the rate of the wind in the center were 75 percent and 50 percent of the amounts at the south periphery. The light intensity at the center was 0.148. Another isolated tree, having a more open crown, showed an increase in the south periphery leaves of about 3 percent and in the center leaves of about 12 percent.

Both the south periphery (22 percent) and center (13 percent) second crop leaves of this tree were thicker than the first crop leaves. The increase in the compactness of the palisade tissue in the south periphery leaves was especially noticeable.

The base leaves of forest individuals were thinner than the center leaves of isolated trees. The light intensity in which these leaves grew was 0.075 and the amount of evaporation 46 percent of that at the apex. The apex leaves were slightly thicker than the south periphery leaves of isolated trees. The thickness of the base leaves was 47 percent that of the apex leaves and the thickness of the palisade in the former was 30 percent that of the latter.

Quercus rubra.—An individual of *Quercus rubra* growing in an *Acer saccharum* and *Tilia americana* forest was studied. The lowest leaves, 7.3 m. high, were 67 percent the thickness of the apex leaves, 12.8 m. high. The difference in the amount of palisade in the leaves from the two positions was not so great as in the trees so far noted. The thickness of the palisade in the base leaves was 57 percent that of the apex leaves. In the base leaves the palisade made up 44 percent; the sponge, 30 percent; the upper epidermis, 16 percent; the lower epidermis, 10 percent of the total thickness. In the apex leaves the palisade made up 52 percent; the sponge, 26 percent; the upper epidermis, 15 percent; the lower epidermis 7 percent.

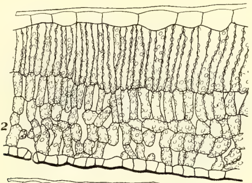
The chief differences in structure were that the apex leaves often had three layers of palisade cells, while the base leaves had two; the palisade was more compact and composed of longer cells, and there was a decrease in the air-space and an increase in the water storage cells and fibro-vascular bundles in the apex leaves.

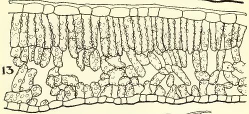
The weight of the green leaves at the base was 63 percent that of the apex leaves, the weight of the water-free leaves 58 percent. The water content of the former was 52 percent, of the latter 49 percent.

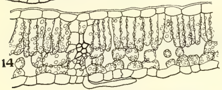
The amount of evaporation at the base was 58 percent that of the apex, and the light intensity at the base was 0.0425.

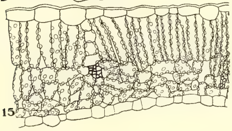
Quercus alba.—The *Quercus alba* studied grew in the forest very near the *Quercus rubra.* The base leaves, 3 5 m. high, were 64 percent the thickness of the apex leaves 9.2 m. high. The thickness of the palisade in the base leaves was 38 percent that of the apex leaves. The thickness of the sponge and the lower epidermis was less in the latter than in the former. In the base leaves the palisade made up 33 percent; the sponge, 41 percent; the upper epidermis, 15 percent;

the lower epidermis, 11 percent of the total thickness. In the apex leaves, the palisade made up 57 percent; the sponge, 25 percent; the upper epidermis, 12 percent; the lower epidermis, 6 percent of the total thickness.









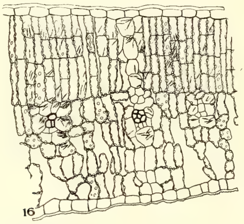
Figs. 12–13. *Quercus macrocarpa*. Fig. 12, Isolated tree. First growth leaves from south periphery. Fig. 13, Figure represents first and second growth leaves from isolated tree, center of crown; and forest tree, base of crown.

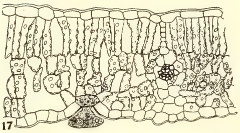
Figs. 14–15. *Acer saccharinum*. Fig. 14, Isolated tree. Leaves from center of crown (Lincoln). Fig. 15, Isolated tree. Leaves from south periphery.

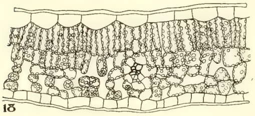
As in the leaves already studied the chief differences in structure consisted in the increase in palisade tissue and the greater compactness of the tissue in the apex leaves. From two to four layers of palisade were found in the apex leaves, while only one was found in the base leaves. The cells in the former leaves were more prolate in shape than those in the latter. The apex leaves had greater bundle development than the base leaves. The weight of the base green leaves was 58 percent, the weight of the water-free leaves 47 percent the weights of the corresponding apex leaves. The water content of the former leaves was 63 percent, of the latter 54 percent.

The amount of evaporation at the base was 57 percent that of the apex, and the light intensity at the base was 0.010.

Acer saccharinum.—Isolated individuals of *Acer saccharinum* were studied in Minneapolis and Lincoln. The center leaves of the tree in the former place were 66 percent the thickness of the south periphery leaves. Most of this increase was caused by the palisade as the thickness of the palisade in the former was but 48 percent that of the latter. In the center leaves the palisade made up 39 percent; the sponge, 32 percent; the upper epidermis, 16 percent; the lower epidermis, 13 percent of the total thickness. In the south periphery leaves the palisade made up 53 percent; the sponge, 26 percent; the upper epidermis, 11 percent; the lower epidermis, 9 percent of the total thickness.

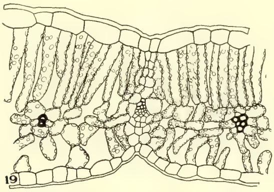


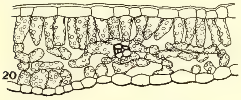


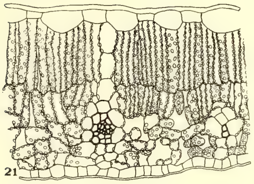


Figs. 16–17. *Fraxinus pennsylvanica.* Fig. 16, Isolated tree. Leaves from south periphery. Fig. 17, Isolated tree. Leaves from center of crown. Fig. 18. *Quercus rubra.* Forest tree. Leaves from base.

The center leaves had one layer of loose palisade while the south periphery leaves had one or two layers composed of larger and more compactly arranged cells. The entire structure of the south periphery leaves was more compact and there was greater development of bundles and water storage cells. The weight of the green center leaves was 60 percent the weight of the south periphery leaves, the weight of the water-free center leaves 42 percent. The water content of the center leaves was 68 percent, of the south periphery leaves 55 percent.







Figs. 19–20. *Platanus occidentalis.* Fig. 19, Isolated tree. Leaves from south periphery (Lincoln). Fig. 20, Isolated tree. Leaves from center of crown (Lincoln).
Fig. 21. *Quercus rubra.* Forest tree. Leaves from apex of crown.

The evaporation at the center was 65 percent that at the south periphery, and the light intensity .038.

The studies of several trees at Lincoln showed that the south periphery leaves of these trees were from 13 percent to 42 percent thicker than the south periphery leaves of Minneapolis trees. The increase was due to the increase of palisade chiefly. The structure of the south periphery leaves from Lincoln was more xerophytic. The center leaves were about the same in both places.

Acer negundo.—The thickness of the center leaves of an isolated *Acer negundo* tree at Minneapolis were 79 percent that of the south periphery leaves. Most of this increase was due to the palisade as it more than doubled in thickness. In the center leaves the palisade made up 33 percent; the sponge, 45 percent; the upper epidermis, 12 percent; the lower epidermis, 10 percent the total thickness. In the south periphery leaves the palisade made up 57 percent; the sponge, 24 percent; the upper epidermis, 11 percent; the lower epidermis, 8 percent of the total thickness. The south periphery leaves had two layers of compact palisade, the center leaves one layer. The amount of evaporation at the center was 67 percent that at the south periphery, and the light was 0.082.

Ulmus americana.—The thickness of the center leaves of an isolated *Ulmus americana* was 64 percent the thickness of the south periphery leaves. The palisade in the former was 45 percent the thickness of that in the latter. In the center leaves the palisade made up 35 percent of the total thickness, the sponge 38 percent, the upper epidermis 16 percent, the lower epidermis 11 percent. In the south periphery leaves the palisade made made up 50 percent of the total thickness, the sponge 26 percent, the upper epidermis 15 percent, the lower epidermis 9 percent.

The south periphery leaves were more compact in structure, the cells were narrower and longer, the upper epidermis more regular, and two layers of palisade were developed as compared with one in the center leaves. The weight of the green center leaves was 71 percent that of the south periphery leaves; the water-free leaves, 55 percent. The water content of the center leaves was 66 percent, of the south periphery leaves 56 percent.

The amount of evaporation at the center was 69 percent; the wind 57 percent the amounts at the periphery. The light intensity at the center was 0.084.

Fraxinus pennsylvanica.—The thickness of the center leaves of an isolated *Fraxinus pennsylvanica* was 63 percent that of the south periphery leaves. The palisade in the former was 38 percent the thickness in the latter. In the center leaves the palisade made up 35 percent the total thickness, the sponge 50 percent, the upper epidermis 8 percent, the lower epidermis 7 percent. In the south periphery leaves the palisade made up 58 percent the total thickness, the sponge 30 percent, the upper epidermis 6.4 percent, the lower epidermis 5.6 percent.

The south periphery leaves were frequently entirely palisaded;

the upper part consisting of three or four layers of very compact cells, the lower part of four or five layers of irregular cells; while in the center leaves there was usually but one layer of palisade. The bundles and water-storage cells, and the crystals were more numerous in the former also.

The weight of the center green leaves was 58 percent that of the south periphery leaves, the weight of the water-free leaves 28 percent. The water content of the former leaves was 67 percent; of the latter, 33 percent.

The amount of evaporation at the center was 53 percent that at the south periphery; the wind, 65 percent. The light at the center was 0.015.

Celtis occidentalis L.—The thickness of the center leaves of an isolated *Celtis occidentalis* at Lincoln was 63 percent the thickness of the south periphery leaves. All of the sponge tissue in the center leaves was palisaded in the south periphery leaves, so the palisade in the former is only 31 percent that in the latter. The differences in structure were again found in the compactness of the cells, the shape of the cells, and the cystolithic cells were more abundant in the south periphery leaves. The light in the center was 0.059.

Platanus occidentalis L.—The thickness of the center leaves of an isolated *Platanus occidentalis* at Lincoln was 61 percent the thickness of the south periphery leaves. The palisade tissue in the center leaves was 46 percent that of the latter. The palisade made up 32 percent the thickness of the center leaves, the sponge 38 percent, the upper epidermis 17 percent, the lower epidermis 13 percent. The palisade made up 42 percent the thickness of the south periphery leaves; the sponge 38 percent, the upper epidermis 12 percent, the lower epidermis 8 percent. The south periphery leaves had more compact tissue, the cells were more prolate, although there was but one layer as in the center leaves. The scalloped appearance of the cross section of the south periphery leaves was caused by the greater bundle and water storage tissue development as compared with the center leaves.

SUMMARY

1. The light intensity, as measured by the Clements photometer, within the crown of 10 common broad-leaved trees was found in August to vary from .0076 of full sunlight in *Acer saccharum* to .1132 in *Quercus macrocarpa.*

2. The evaporation, measured by the Livingston porous cup atmometers, was found to be from 1½ to 2⅓ times as great at the south periphery as within the crown.

3. The temperature at the south periphery was usually but one or two degrees higher than within the crown.

4. The humidity, measured by cog-psychrometers, was usually from 1 percent to 6 percent higher within the crown.

5. A wind of low velocity caused greater differences in the air movement between the center and the periphery of the crown than a strong wind. The wind was found to be from 1⅓ to 8 times as strong at the periphery as within the crown.

6. Transpiration experiments showed that the south periphery leaves lose more water per unit area than the center leaves. In *Fraxinus pennsylvanica* the south periphery leaves lost from 3 to 6 times as much as the center leaves; in *Ulmus americana* about 12 times as much. Even when the potometer containing south periphery leaves is placed under similar conditions with the potometer containing center leaves it will lose more water per unit area.

7. The leaves from the periphery of the tree were usually more deeply lobed, more prominently toothed, and smaller than the leaves from the center of the same tree.

8. The water content of the leaves from the center of the tree was always higher than that of the leaves from the south periphery. The amount of dry material per unit area in the exposed leaves bears a relation to tolerance. The dry weight of the leaves of the most tolerant trees is less per unit area than the dry weight of the leaves of the least tolerant trees, as, leaves from *Acer saccharum* contain 1.029 gr. of dry matter per unit area, while leaves from *Quercus macrocarpa* contain 1.272 gr.

9. *The differences in the total thickness between the south periphery and the center leaves on the same tree are usually greater than the differences heretofore reported from leaves of mesophytic and xerophytic forms of the same species.* The leaves from the south periphery have more palisade tissue, greater compactness of structure, thicker epidermis and cuticle than the leaves from within the crown.

This subject, the structural response of leaves of the same plant to measured environmental factors, is so large that this paper can only be considered as an opening wedge into further investigation. Detailed studies are needed on specific aspects, as transpiration, water content, etc.

In conclusion I wish to acknowledge my indebtedness to Dr. J. E. Weaver, who suggested this investigation and assisted in securing a part of the data obtained during the summers of 1915 and 1916. I also wish to express my appreciation of the encouragement offered by Dr. Raymond J. Pool and of the facilities afforded by the department of botany at the University of Nebraska.

The University of Nebraska

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CONTENTS

The phytogeography of Manoa Valley, Hawaiian Islands. VAUGHAN MACCAUGHEY 561
Revision of the Hawaiian species of the genus Cyrtandra, section Cylindrocalyces Hillebr. .... JOSEPH F. ROCK 604
On the distribution of abnormalities in the inflorescence of Spiraea Vanhouttei. J. ARTHUR HARRIS 624
Index to Volume IV 637

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THE PHYTOGEOGRAPHY OF MANOA VALLEY, HAWAIIAN ISLANDS

VAUGHAN MACCAUGHEY

The present paper represents the first effort, in the long history of botanical exploration in the Hawaiian Archipelago, to make a detailed ecological survey of a representative Hawaiian phytogeographic area. Taxonomic lists and descriptions of new species comprise the bulk of the scientific literature dealing with the Hawaiian flora, and in all of this material there is a conspicuous absence of physiographic and ecologic data. The present paper is based upon field observations extending over a residence of nine years on the island of Oahu, of which time four years have been passed in Manoa Valley itself. The writer has repeatedly visited all portions of this beautiful and historic valley, and has conducted many collegiate field excursions to the numerous points of prime botanic interest.

The writer has availed himself of all accessible records. The nomenclature followed has been chiefly that of Hillebrand's monumental Flora of the Hawaiian Islands (1888). Although this nomenclature is somewhat obsolescent, it is in common usage in the island literature, and it was deemed inadvisable to cumber too greatly these pages with revised names of familiar plants. In numerous instances however, the modern taxonomy has been introduced.

The College of Hawaii is situated in Manoa Valley, near Honolulu. This valley is the immediate natural background of the College and its botanical instruction. Manoa is a representative ecologic area of the Hawaiian mountains. It presents a very clearly defined series of life zones, both in vertical and horizontal planes. It is typical of many valleys in the Hawaiian Islands, and in other parts of the Polynesian Pacific. The phytogeography of Manoa Valley epitomizes that of any similar physiographic region in the archipelago.

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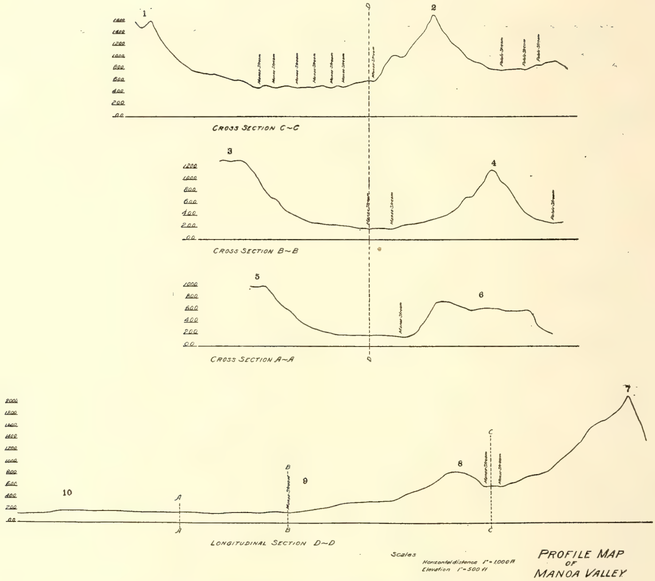
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Scales
Horizontal distance 1" = 1,000 ft
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PROFILE MAP OF MANOA VALLEY

FIG. 1. *Vertical Cross and Longitudinal Sections of Manoa Valley.*

1. West lateral ridge; transition region; steep wall into hygrophytic valley-head.

2. East lateral ridge; mountainward portion, covered with rain-forest; eastward slope is into the head of Palolo Valley.

3. West lateral ridge; Mount Tantalus; sloping into middle valley floor.

4. East lateral ridge; apex of foothill.

5. West lateral ridge; Roundtop; sloping into lower valley floor.

6. East lateral ridge; Manoa-Palolo Foothill, about midway between plain and apex.

7. Summit Ridge, near Mount Olympus.

8. Puu Pueo, a median ridge lying along the central axis of the valley.

9. Middle portion of the valley floor, near the point of union of the main tributaries of Manoa Stream.

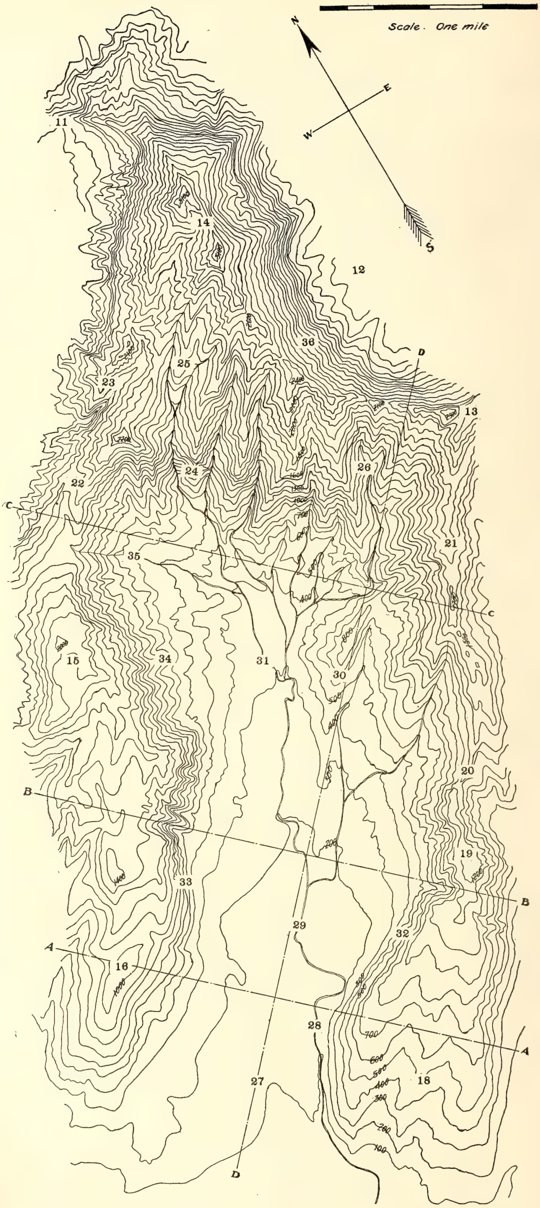
10. The lower valley floor.

Manoa is situated on the island of Oahu, in the vicinity of the city of Honolulu. Residential districts lie along portions of the mouth of the valley and lower western slopes. Much of the floor is occupied by agricultural lands—*taro*, bananas, vegetable gardens, etc. Oahu is third in size among the Hawaiian islands. It is 46 miles long and 25 miles wide, with an area of 598 square miles. It is topographically distinguished from the other islands by being composed of two elongate mountain ranges, Waianae and Koolau. These are of great antiquity, deeply eroded, and give evidence of numerous and extensive elevations and subsidences.

The Waianae Range, lying on a NW.–SE. axis, is about 20 miles long. Its highest peak, Ka-ala, is 4,030 feet high; this is the highest point on the island. The highest point in the archipelago is Mauna Kea, on Hawaii, 13,825 feet. The Koolau Range, in which Manoa is carved, lies to the northeast of the Waianaes, parallel with the latter, at a mean distance of eighteen miles. The Koolau Range is 37 miles long, and is the longest range in the archipelago. It is low, its mean elevation not exceeding 2,000 feet. The highest peak, Kona-hua-nui, rises to 3,105 feet, and lies at the head of Manoa Valley. The range is deeply sculptured by subaerial erosion. There are about fifty major valleys, with numberless ravines and lateral gullies. Manoa is one of the largest of the major valleys.

Manoa is a well-matured valley, with broad flat floor and slightly expanded head. Measured from its mouth or portal (using the 100 feet contour as a base-line), an airline to the crest of the summit ridge is 3.4 miles long. Its width, measured by airline from one lateral ridge-crest across to the opposite ridge-crest, varies from 1.2 miles at the portal to 2.2 miles at the head. Like many other of the larger Hawaiian valleys—Kalihi, Kahana, Iao, Pelekunu, Halawa, Waipio—the head of Manoa is a constantly expanding amphitheater of erosion. The valley widens progressively from portal to head, at the rate of about 5–6 percent.

The Koolau Range lies along a NW.–SE. axis. All the valleys, of which Manoa is one, that deeply furrow its leeward flanks have a dominant southwesterly exposure. The trade winds, which blow almost continuously through a major portion of the year, come from the northeast. The leeward valleys are thus protected from the trade winds by the mountain wall. The maximum of the torrential precipitation that results from the rising of the moisture-laden trades over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the mountain rampart falls, not upon the summit crest, but just to the leeward. The heads of the leeward valleys thus receive Oahu's maximum precipitation. The annual average for this is about 150 inches, whereas the precipitation along the crest itself is about 100 inches. Manoa is known locally as a very rainy valley.

Rainfall has been a dominant factor in forming the valley and sculpturing its walls. At present it is the controlling factor in the distribution of the plant life of the valley. The following data, sup-

Locality	Jan.	Feb.	Mch.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Annual (Approximate)
Honolulu, on coastal plain, el. 111 ft.	18	3	7	2	2	1	2	1	1	1	1	2	41
Manoa, middle of valley, el. 300 ft.	7	7	8	8	7	6	7	8	8	7	9	9	90
Manoa, upper floor, el. 300 ft.	22	3	11	9	28	12	13	19	9	9	17	24	176
Mount Tantalus, el. 1,360 ft.	8	8	9	9	8	8	9	8	9	8	11	11	106

FIG. 2. *Contour Map of Manoa Valley*

1. Nuuanu Valley Gap; at the head of the valley, cutting completely through the Koolau Range.

12. Ohu-ohi Amphitheater, on the windward side of the Koolau Range.

13. Mount Olympus.

14. Mount Kona-hua-nui, with two summit peaks.

15. Mount Tantalus.

16. Round top Hill; Sugar-Loaf lies directly mountainward, between the former and Tantalus.

18. The Manoa-Palolo foothill.

19. Apex of the Manoa-Palolo foothill.

20. Transition region on the east lateral ridge.

21. Mountainward portion of the east lateral ridge.

22. Topographic transition region on the west lateral ridge.

23. Mountainward portion of the west lateral ridge.

24. Zone of precipices or *palis* that bound the upper valley head.

25. Zone of the hanging valleys; in addition to the five or six large hanging valleys there are numberless small ones.

26. Hanging valleys in the flanks of Mount Olympus.

27. The lower valley floor.

28. Manoa stream, crowded against the foot of the east ridge.

29. The middle portion of the valley floor.

30. Puu Pueo.

31. The upper valley floor.

32, 33, 34, 35. Talus zone and valley walls.

36. Windward wall or precipice of the Koolau range.

plied by the Hawaii Section, U. S. Weather Bureau, shows the rainfall in various parts of the valley, for 1916.

Translating these data into terms of ecologic zones, the approximate annual rainfall is as follows:

Coastal plain, seaward of Manoa valley	41 inches
Middle of valley	90 "
Upper valley floor	176 "
Lower forest zone	106 "

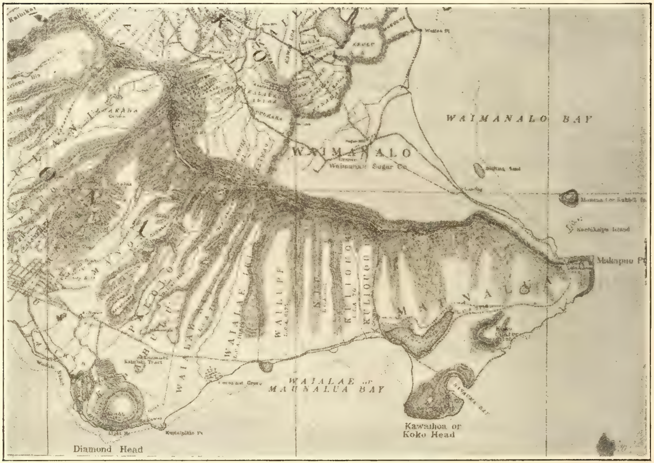
WAIMANALO BAY
WAIMANALO
Waimanalo Sugar Co.
Makapuu Pt
WAILUPE
KULIOUOU
NIU
WAIALAE
WAIALAE or MAUNALUA BAY
Kawaihoa or Koko Head
Diamond Head

FIG. 3. Map of the eastern end of Oahu showing the relation of Manoa valley to the adjacent land areas.

In general the valley becomes progressively hygrophytic as one advances toward the head, and conversely, progressively xerophytic as one approaches the sea.

In Luakaha, a region in upper Nuuanu Valley, and separated from Manoa by only a narrow ridge, the annual rainfall is about 200 inches (196.99).

The U. S. Hygrographic Survey maintained a rain-gauge on the summit of Mt. Olympus (2,450 feet) for a period of 67 days, and recorded a daily rainfall of .20 inch. This would be equivalent to an annual precipitation of about 73 inches, a figure somewhat lower than the general average for the rain-forest.

Shreve[1] gives the annual rainfall for three stations in the Jamaica rain-forest as 105.70, 113.85, and 168.02 inches respectively. This corresponds closely with records for the Hawaiian rain-forests, as does his statement that "there is no other form of precipitation than rain, hail and snow being unknown, although the former occurs at rare intervals in the lowlands. The frequency of showers too light to register 0.01 inch is high, and they are not without influence on vegetation. Although the number of rainy days is high and the frequency of light showers is high, yet the bulk of the annual rainfall is registered during the prolonged downpours. . . . Dew is formed abundantly in open situations on clear nights at all seasons of the year."

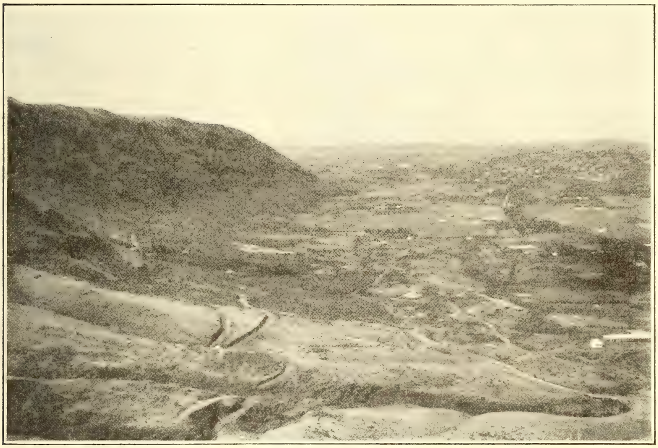

FIG. 4. View of Manoa Valley, from a lateral ridge. Shows plainly the lower floor, talus zone, wall and eastern foot hill.

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[1] Shreve, Forrest, A Montane Rain-Forest. Carnegie Institution of Washington, 1914.

Although somewhat sheltered from the direct mechanical effects of the trades, Manoa and the other leeward valleys of its class are exposed to the periodical *kona* or southerly storms, which usually occur during the late winter and early spring (January to April). The kona storms are often characterized by heavy winds and excessive rainfall.

The southwesterly exposure of Manoa shuts off its head from a considerable portion of the morning sunlight, and gives prominence to the afternoon heat and light. Manoa is much sunnier and warmer than are the narrow, windy, northerly facing valleys of the windward Koolau slopes. This climatic difference is sufficiently great to be reflected in the respective floras of these two types of valleys.

I. THE REPRESENTATION IN MANOA OF THE HAWAIIAN ECOLOGIC ZONES

In the Hawaiian Archipelago there are numerous well-defined ecologic zones. The representation of these life-strata in the Manoa region may be indicated as follows:

1. Littoral. *a. Humid littoral;* windward.

   b. Arid or semi-arid littoral; leeward. The littoral of that portion of the coastal plain which lies to the seaward of Manoa Valley is of this type.
2. Lowlands. Up to 1,000–1,500 feet; with humid and arid sections, depending upon relation of topography to trade winds, and distance from interior mountains. In Manoa Valley the lowland proper (valley floor) lies well below the 500-foot contour; in early times the lower forest zone came down to this level.
3. The Forest Zone. *a. The Lower Forest;* 1,000–2,000 feet; with humid and arid sections. In Manoa this zone lies between 500 and 1,200 feet, and is almost wholly of hygrophytic or semihygrophytic character, although some xerophytic forms do occur.

   b. The Middle Forest; 1,800–5,000 feet; variable with humid and arid sections on the various islands. In Manoa this zone is typical Oahuan rain-forest; highly hygrophytic, and very rich in endemic forms. Owing to the low elevation of the Oahu mountains, this is the highest zone, and

   c. The Upper Forest; 5,000–9,000 feet, is restricted to the high mountains of Maui and Hawaii.

4. THE SUMMIT REGIONS. *a. Xerophytic Summits;* 9,000–14,000 feet; high mountains of Maui and Hawaii.

   b. Hygrophytic Summits; 4,000–6,000 feet; peaks rising into the cloud zone, Kauai, Waianae, East Molokai, West Maui, Kohala.

2. ECOLOGIC ZONES OF MANOA

The main ecologic zones in Manoa Valley are:

1. The Valley Floor:
   - *a.* The Lower Floor (near portal).
   - *b.* The Upper Floor (near head).
2. Manoa Stream and its Tributaries.
3. The Talus Zone.
4. The Valley Walls or Lateral Ridges:
   - *a.* The East or Manoa-Palolo Ridge,
     1. Foothill.
     2. Transition region.
     3. Mountainward region.
   - *b.* The West or Mt. Tantalus Ridge,
     1. Foothill (Roundtop).
     2. Transition region.
     3. Mountainward region.
5. The *Kukui* Zone, Ravines, and Precipices.
6. The Zone of *Koa* and *Lehua.*
7. The Hanging Valleys; Rain Forest.
8. Summit Ridges and Peaks:
   - *a.* Olympus.
   - *b.* Kona-hua-nui.

Topographic, edaphic, climatic, and biotic factors differentiate more or less clearly these zones from one another. On the basis of *water*, the grouping would be, as numbered above:

Hydrophytic	2.	Mesophytic	1*a*, 3.
Hygrophytic	1*b*, 4*a*23, 4*b*23, 5, 6, 7, 8.	Xerophytic	1*a*, 3, 4*a*1, 4*b*1.

On the basis of *elevation:*

Above 2,000 ft.	8*a* and *b*.		
Between 1,000–3,000	7.	Between 1,200–1,700	4*a*2, 4*b*2.
1,000–1,400	6.	1,000–2,000	4*a*3, 4*b*3.
300–1,000	5.	100–300	3.
50–1,000	4*a*1, 4*b*1.	50–300	1, 2.

On the basis of *mean temperatures:*

Notably warm	1, 4*a*1, 4*b*1.	Cool	4*a*2, 4*b*2, 5.
Warm	3.	Notably cool	4*a*3, 4*b*3, 6, 7, 8.

3. THE VALLEY FLOOR

The floor of Manoa is conspicuously broad and flat, much more so than are the floors of the valleys immediately adjacent to it, Makiki, Pauoa, Palolo, and Waialae. This flatness may be considered as one of the evidences of the maturity of this valley.

The designation "floor" comprehends the region lying below the 300-foot contour; roughly an area 2.0 miles by .75 mile. It is composed chiefly of "mountain wash," a heavy, dark reddish-brown, fine-textured, adobe soil, that has been washed down from the surrounding basaltic ridges and spread out as a deep blanket in the valley basin. Along the lower western slopes are extensive deposits of volcanic ash and cinders. The thickness of the soil bedding is not known; along the center of the valley it must be very deep, perhaps hundreds of feet. The red-brown adobe soil is fertile, stiff and intractable in cultivation, and exceedingly retentive of moisture. When it becomes dry to any considerable depth, as during the infrequent droughts, it cracks conspicuously. The cracks are 1 to 4 inches wide and 12 to 40 inches deep.

From an ecologic standpoint the valley floor may be transversely divided into two regions, the floor of the lower valley, *i. e.*, near the portal, and the floor of the upper valley, near the head. The lower floor comprises the area from the portal up to the point at which Manoa Stream diverts from the middle of the valley. The upper floor continues from this latter point to the region beyond the bifurcation of the floor at Puu Pueo. The lower floor is contrasted with the upper floor by greater xerophytism; more brilliant illumination; higher temperatures of air and soil; less surface water in the form of streams, pools, and springs (although more in the form of irrigated *taro* patches); more volcanic material such as surface lava, cinders, ashes, etc.; and smoother contours. The upper floor has a higher percentage of indigenous vegetation, and in former times was wholly covered by the lower forest zone, as will be described in another section.

The valley floor is principally occupied by introduced plants, including both weeds and economics. The native vegetation is poor in species and quantitatively insignificant. This condition is in striking contrast with that of the rain-forest, only a few miles distant, where the vegetation is almost wholly endemic or indigenous, and where the introduced element is practically negligible.

Some of the larger and dominant plants of the valley floor (aside from those actually under cultivation), are: *Prosopis juliflora*, *Opuntia megacantha*, *Leucaena glauca*, *Lantana Camara*, *Psidium Guayava*, *Xanthium strumarium*, *Ricinus communis*, *Indigofera Anil*, *Malvastrum tricuspidatum*, Cassia spp., Sida spp., *Acacia Farnensiana*, Ipomoea spp., *Commelina nudiflora*, Crotalaria spp., *Eugenia Jambolana*, *Stachytarpheta dichotoma*, *Solanum Sodomeum*, etc.

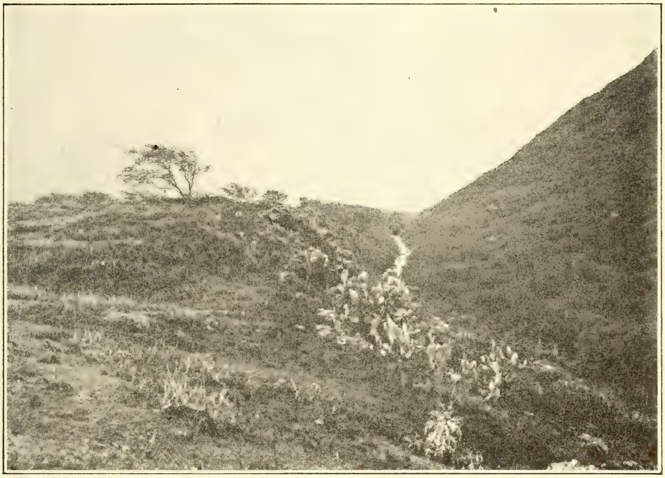

FIG. 5. View of Manoa stream and east valley wall. Looking toward head of valley, which may be seen faintly through the rain. Trees in mid-ground are *Kiawe*, *Prosopsis juliflora*.

Manoa has been inhabited by the native Hawaiians since very early times. Much of the lower floor was occupied by their tiny plantations or *kuleanas*. The *kalo* or *taro* (*Colocasia antiquorum*

Schott) was the principal crop, and was raised in small irrigated fields or *loi*. The water from these fields was skilfully diverted from Manoa stream by a primitive but highly efficient system of ditches. An area equivalent to several square miles was occupied by the *kalo* fields. Much of this *kalo* land is in cultivation today, although the industry has passed largely into the hands of Orientals.

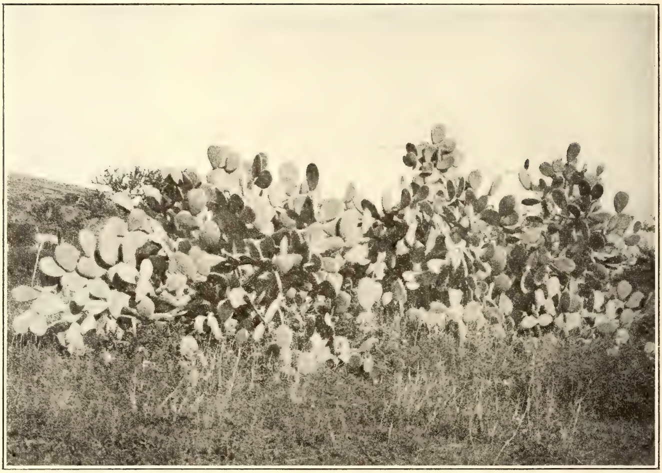

FIG. 6. *Opuntia megacantha*, a dominant xerophyte of the Manoa lower valley floor and foot hills.

Other crops raised by the primitive Hawaiians, and continuing today in small patches here and there, are

Sweet potato	*Uala*	*Ipomoea Batatas*
Native banana	*Maia*	*Musa sapientum*
Sugar cane	*Ko*	*Saccharum officinarum*

Mention may be made of the eleven avian species that are distinctive of the valley floor and walls. Six of the introduced species are common and of considerable phytogeographic significance, as they are abundant carriers of weed seeds and fruits.

4. BIRDS OF THE VALLEY FLOOR

Endemic

Short-eared owl, *Asio accipitrinus Sandwichensis* Bloxam. *Pueo*	R
Hawaiian coot, *Fulica alai* Peale. *Alae keokeo*	R
Hawaiian gallinule, *Gallinula galeata Sandwicensis* Streets. *Alae ula*	R

Indigenous

Black-crowned night heron, *Nyctocorax nyctocorax griseus* Bodd.	F

Naturalized

Rice bird, *Munia nisoria punctata* Temm.	C
English sparrow, *Passer domesticus* L.	C
Mynah bird, *Acridotheres tristis* L.	C
Skylark, *Alauda arvensis* L.	F
Chinese reed warbler, *Trochalopterum canorum* L.	C
Chinese turtle dove, *Turtur Chinensis* Scop.	C
Australian alae, *Alae iwi, Porphyrio melanotus* Newton	R

Explanation of Symbols

H—herbaceous; annual.
S—shrubby.
A—arborescent.
P—perennial.
V—liana habit.
C—common.
F—frequent.
R—rare.

5. REPRESENTATIVE PLANTS OF THE VALLEY FLOOR

Group I. Endemic

I*a. Hydrophytes*

Hibiscus Youngianus Gaud.	HSF

I*b. Mesophytes*

Nama Sandwicensis Gray	HF
Sicyos cucumerina Gray	HR
" *pachycarpa* Hook. & Arn.	HR
Solanum aculeatissimum Jacq.	SF

I*c. Xerophytes*

Abutilon incanum G. Don.	HSF
Chenopodium Sandwicheum Moq.	HSF
Erythraea sabaeoides Gray	HF
Jacquemontia Sandwicensis Gray	HVF

I*d. Parasites*

Cuscuta Sandwichiana Chois.	HVF

I*e. Pteridophytes*

Ophioglossum concinnum Brack.	F

Group II. Indigenous

II*a. Hydrophytes*

Commelina nudiflora L.	HPC
Kyllingia monocephala Rottb.	HPC

II*b. Mesophytes*

Andropogon contortus Roem. & Schult.	HPF
Caesalpinia Bonducella Fleming.	VSPF
Chrysopogon aciculatus Trin.	HPC
Cyperus pennatus Lam.	HPC
Ipomoea bona-nox L.	HPVF
" *pentaphylla* L.	HVC
Nicandra physaloides Gaertn.	HF
Panicum pruriens Trin.	HPC
Plumbago Zeylanica L.	SPF
Wikstroemia foetida var. *Oahuensis* Gray	SPF

II*c. Xerophytes*

Boerhaavia diffusa L.	HSF
Eleusine Indica Gaertn.	HPC

Erythrina monosperma Gaud.	AR
Ipomoea tuberculata Roem. & Schult.	HPVC
Panicum torridum Gaud.	HC
Sida fallax Walp.	HSC
" *rhombifolia* L.	HSC
Tephrosia piscatoria Pers.	SPF
Tribulus cistoides L.	SPF
Waltheria Americana L.	SC

II*d*. *Pteridophytes*

Ceropteris calomelaena Link.	C
" *ochracea* Robins.	C
Dryopteris propinqua Gilb.	C
Microlepia strigosa Presl.	C
Nephrolepis cordifolia Presl.	F
" *exaltata* Schott.	C
Odontosoria Chinensis J. Sm.	C

GROUP III. INTRODUCED BY THE PRIMITIVE HAWAIIANS

III*a*. *Hydrophytes*

Alocasia macrorrhiza Schott.	HPF
Colocasia antiquorum Schott.	HPC

III*b*. *Hygrophytes*

Artocarpus incisa L.	AC
Eugenia Malaccensis L.	AF
Musa sapientum L.	HPC

III*c*. *Mesophytes*

Calophyllum inophyllum L.	AF
Cocos nucifera L.	AC
Cucurbita maxima Duch.	HVR
Hibiscus tiliaceus St. Hil.	AC
Ipomoea Batatas L.	HPVC
Lagenaria vulgaris Ser.	HVR
Saccharum officinarum L.	HPC
Thespesia populnea Correa	AF

III*d*. *Xerophytes*

Morinda citrifolia L.	AC
Pandanus odoratissimus L.	AC

GROUP IV. PLANTS NATURALIZED SINCE THE ADVENT OF EUROPEANS

IV*a*. *Hydrophytes*

Arundo Donax L.	HPF
Coix lachryma L.	HPC
Panicum barbinode Trin.	HC
Sagittaria sagittifolia L.	HC

IV*b*. *Hygrophytes*

Coffea arabica L.	AF
Eugenia Jambos L.	AC
Physalis Peruviana L.	HPC

IV*c*. *Mesophytes*

1. Trees and Shrubs

Bixa Orellana L.	F
Carica Papaya L.	F
Cassia chamaecrista L.	F
" *laevigata* Willd.	F
" *occidentalis* L.	C
Cestrum diurnum L.	F
Citrus spp.	F
Eugenia Jambolana Lam.	C
Jatropha Curcas L.	R
Leucaena glauca Benth.	AC
Melia Azedarach L.	AC
Mimosa pudica L.	HPSR
Mammea Americana L.	AF
Psidium Cattleyanum Sab.	AC
" *Guayava* L. and vars.	ASC
Ricinus communis L.	SAC
Spondias dulcis L.	AF
Terminalia Catappa L.	AC

2. Herbaceous Perennials

Bambusa vulgaris Schrad. & Wendl.	F
Canna Indica L.	C
Cajanus Indicus Spreng.	C
Crotalaria fulva Roxb.	C
" *saltiana* Andr.	C
" *spectabilis* Roth.	C
Cynodon Dactylon Pers.	C
Cyperus rotundus L.	C

Echinochloa Crus-galli (L.) Beauv.	C
Medicago apiculata Willd.	F
“ *denticulata* Willd.	F
“ *Indica* All.	F
“ *intertexta* Mill.	F
“ *lupulina* L.	C
Mirabilis Jalapa L.	C
Panicum barbinode Trin.	C
Paspalum conjugatum Berg.	C
Taraxacum officinale Weber	R
Thunbergia alata Boj. and var. *aurantiaca* Ktz.	F
Verbena Bonariensis L.	C

3. Annual Herbs

Asclepias Curassavica L.	F
Bothriospermum tenellum F. & M.	F
Brassica campestris L.	R
Cuphea hyssopifolia H. B. K.	R
Erigeron albidus Gray	F
“ *Canadensis* L.	C
Euphorbia geniculata Ort.	C
“ *pilulifera* L.	C
Fleurya interrupta Gaud.	R
Franseria tenuifolia Gray	F
Malva rotundifolia L.	C
Oxalis corniculata L.	F
“ *Martiana* Zuccar.	C
Peucedanum graveolens Benth. & Hook.	F
Plantago major L.	R
Siegesbeckia orientalis L.	F

4. Vines

Abrus precatorius L.	F
Basella rubra L.	R
Cardiospermum Halicacabum L.	C
Clerodendron fragrans Vent.	F
Clitoria Ternatea L.	F
Convolvulus spp.	C
Dolichos Lablab L.	C
Ipomoea chryseides Ker-Gaul.	F
Paederia foetida L.	C
Passiflora foetida L.	F

IV*d. Xerophytes*

1. Trees and Shrubs

Acacia Farnensiana Willd.	C
Cassia occidentalis L.	C
Casuarina equisetifolia L.	F
Eucalyptus spp.	C
Indigofera Anil L.	C
Nicotiana glauca Grah.	F
Opuntia megacantha Salm.	C
Phoenix dactylifera L.	F
Prosopis juliflora L.	C

2. Herbaceous Perennials

Abutilon spp.	C
Amaranthus spinosus L.	C
Desmodium uncinatum DC.	C
Drymaria cordata Willd.	F
Momordica charantia L.	FV
Phaseolus semierectus L.	C
Priva aspera H. B. K.	R
Sida spinosa L.	F
Solanum Sodomeum L.	C
Stachytarpheta dichotoma Vahl.	C
Xanthium Strumarium L.	C

3. Annual Herbs

Ageratum conyzoides L.	C
Anagallis arvensis L.	F
Bidens pilosa L.	C
Chaetochloa verticillata Scribn.	C
Chenopodium album L.	C
“ *hybridum* L.	E
“ *murale* L.	F
Datura Stramonium L.	F
Eleusine Aegyptiaca Pers.	C
“ *Indica* Gaertn.	C
Emilia flammea Cass.	R
Erodium cicutarium L'Her.	F
Euxolus viridis Moq.	F
Gynandropsis pentaphylla DC.	C
Malvastrum tricuspidatum Gray.	C
Portulaca oleracea L.	C
Rumex Acetocella L.	F
Senebiera didyma Pers.	F
Sonchus oleraceus L.	C
Stachys arvensis L.	F
Vernonia conyzoides L.	C

It will be noted that this list, which includes practically all of the important species of this region, comprises the following groups:

	Species
Endemic	11
Indigenous	29
Introduced by primitive Hawaiians	15
Introduced since the advent of Europeans	115

Hydrophytes	9
Hygrophytes	6
Mesophytes	82
Xerophytes	56
Vines or lianas	18
Pteridophytes	8

6. MANOA STREAM

The surface drainage waters of Manoa escape as a single small and fluctuating brook, known as Manoa Stream. A very considerable percentage of the Manoa drainage makes its way to the sea through subterranean channels; this is a condition universal throughout the islands. Manoa Stream is fed by numerous tributaries, which enter

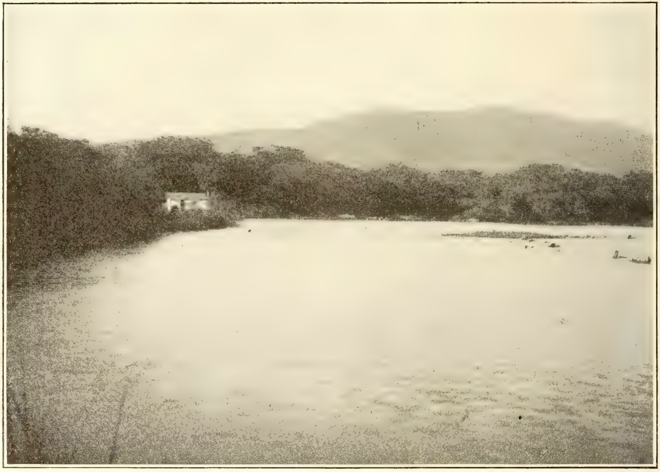

FIG. 7. Manoa stream near its mouth. The trees are *Prosopsis juliflora*. In the distance is the west lateral ridge, with Round Top and Tantalus showing distinctly.

the head of the valley over beautiful waterfalls. There is also a series of springs along the foot of the cliffs at the head of the valley, which contribute their waters to the stream. Other springs, notably those on the grounds of the Kawaiahao Seminary and Punahou Academy, occur on the valley floor.

The ten contributory branches of the stream are freshet in character and very intermittent in their flow. The stream proper is never wholly dry. After the rare protracted droughts it becomes very low, and its branches cease to flow. The upper course of the stream lies in the middle of the valley. The lower course has been strongly deflected against the east foothill, presumably by the outpourings of lava and other volcanic material from the craters in the west foothill. The stream leaves the valley at the extreme eastern side, hugging the base of the east foothill, where it has carved a small narrow canyon through the thick beds of ancient flow lava.

The stream is marked throughout its course by vegetation characteristic of streamways and swampy places. Due to the general utilization of the stream waters for irrigation, the swampy areas and *loi kalo* (*taro* patches), adjacent to the stream itself are here considered as a part of this ecologic zone. The vegetation of the streamway is nowhere sharply differentiated from that of the valley floor. In numerous instances the species that grow most luxuriantly along the stream are also forms most abundant on the valley floor.

From the ecologic standpoint the stream is at present a factor of minor importance in determining the phytogeography of Manoa. It undoubtedly had a more prominent rôle in early times, before the valley floor was overrun by introduced vegetation. One of the influences of the stream is as an agent for the dissemination of seeds. Frequently the seeds of montane species are carried to lower levels, where they occasionally establish themselves. It is extremely significant, however, that there has been no general seaward migration of montane species via the stream; in general the forests have retreated up stream.

7. PLANTS ABUNDANT ALONG OR CHIEFLY CHARACTERISTIC OF THE MANOA STREAM AND ITS TRIBUTARIES, INCLUDING ADJACENT SWAMPS AND TARO-PATCHES

Group I. Algae

Anabaena confervoides Reinsch.
“ *variabilis* Kuetz.
Aphanothece repens A. Br.
Bulbochaeta spp.
Calothrix fusca Bornet.

Chamaesiphon curvatus Nordst.
Chara coronata var. *leptosperma.*
" *forma Oahunesis* A. Br.
" *gymopus* var. *armata* Nordst.
Cladophora Nordstedii De T.
Closteriopsis longissima Lemm.
Coleochaete irregularis Pringsh.
" *orbicularis* Pringsh.
Conferva bombycina var. *minor* Wille.
" *Sandwicensis* Ag.
Dactylococcus infusionum var. *minor* Nordst.
Dictyosphaerium pulchellum Wood.
Draparnaldia macrocladia Nordst.
Gloeothece fuscolutea Naeg.
Gonium sociale Warm.
Hydrodictyon reticulatum Lagerh.
Lyngbya aestuarii Liebman.
" *distincta* Schm.
" *rivularium* Gomont.
Merismopedium glaucum Naeg.
Nitella Havaiensis Nordst.
Nostoc commune Vaucher.
" *paludosum* Kuetz.
" *piscinale* Kuetz.
" *punctiforme* Hariot.
Raphidium polymorphum Fres.
Rivularia natans Welw.
Scenedesmus quadricauda Breb.
Scytonema crispum Bornet.
Spirogyra spp.
Stigeoclonium Falklandicum Kuetz.
Stigonema aerugineum Tilden.
" *ocellatum* Thuret.
Ulothrix minulata Kuetz.
" *subtilis* Kuetz.
Xenococcus Kerneri Hansg.
Zygnema spontaneum Nordst.

GROUP II. PTERIDOPHYTES

Marsilea villosa Kaulf.	F, endemic.
" *crenulata* Desv.	R, "
Azolla sp.	F, recent introduction.

GROUP III. SPERMATOPHYTES

IIIa. *Indigenous*

Aster divaricatus Torr. & Gray	HPF
Bidens chrysanthemoides Michx.	HF
Cladium leptostachyum Nees & Meyen	HPL
Commelina nudiflora L.	HPC
Cyperus auriculatus Nees.	HPL
" *laevigatus* L.	HPF
Eleocharis obtusa Schultes	HPL
Ipomoea bona-nox L.	HPVF
" *reptans* Poir.	HPVF
Jussiaea villosa Lam.	HPC
Kyllingia monocephala Rottb.	HPC
Naias major All.	HC
Polygonum glabrum Willd.	HF
Poa annua L.	HF
Potamogeton fluitans Roth.	HPF
" *pauciflorus* Pursh	HPF
Scirpus lacustris L.	HPF
" *maritimus* L	HPR
Zingiber Zerumbet Ros.	HPF

IIIb. *Introduced by the Primitive Hawaiians*

Aleurites Moluccana Willd.	AF
Alocasia macrorrhiza Schott	HPF
Colocasia antiquorum Schott	HPC
Eugenia Malaccensis L.	AF
Hibiscus tiliaceus L.	AC
Musa sapientum L.	HPF

IIIc. *Introduced Since the Advent of Europeans*

Coix lachryma L.	HPC
Canna Indica L.	HPC
Cyperus rotundus L.	HPC
Echinochloa colonum Link	HPC
Eugenia Jambos L.	AC
Hydrocotyle Asiatica L.	HC
" *verticillata* Thunb.	HC
Leucaena glauca Benth.	SC
Lemna minor L.	HC
Nasturtium officinale R. Br.	HF
Mazus rugosus Lour.	HF
Panicum barbinode Trin.	HC
Pithecolobium Saman	AF
Psidium Guayava L.	SC
Sagittaria sagittifolia L.	HC

8. THE TALUS ZONE

Between the valley floor and the valley walls lies an intermediate physiographic and floristic zone, which may be designated as the talus zone. This zone comprises, as its name indicates, the talus deposits at the foot of the valley wall and resting upon the floor. It varies in width from approximately 100 to 1,000 feet. The slope averages 10–15°, as contrasted, on the one hand, with the 40° slope of the wall, and, on the other, with the nearly flat floor. The soil of the talus zone varies considerably in nature in various parts of the valley—in some places it is fine-grained lava soil; in others, coarse volcanic ash and cinders; and in others the ground is littered with massive boulders that have been dislodged from the heights above. It is probable that the talus slope is not, in cross-section, wholly composed of talus; the surface layers are of debris, and below them are the ancient lava-sheets of the valley walls. This situation is revealed by the little streamways that are cut through the talus zone.

The dominant plant of the Manoa talus zone is the guava, *Psidium Guayava.* Secondary species are: *Lantana Camara*, *Paspalum conjugatum*, *Andropogon contortus*, *Verbena Bonariensis*, *Psilotum nudum*, *Morinda citrifolia*, *Nephrolepis exaltata*, *Solanum sodomeum*, Convolvulus spp., *Cassia occidentalis*, *Opuntia megacantha*, *Waltheria Americana*, etc. The talus zone, like the valley floor upon which it rests, is covered almost exclusively with ruderal vegetation. Arborescent forms are infrequent; vigorous and drought-resistant herbaceous-woody shrubs are the prevailing types.

In primitive times the talus zone of the upper valley was completely clothed with native trees, the species being those of the lower forest zone. The forest retreated before the incursions of man and wild live-stock, and exposed the talus zone to the invasions of foreign vegetation. The hilo grass (*Paspalum conjugatum*) has been notably pernicious, as it forms a dense sod and effectually prevents the native species from reseeding themselves.

The talus zone of the lower valley probably has been always more or less xerophytic in character. Many of the indigenous or endemic xerophytes of Hawaii have become extinct or are now upon the verge of extinction. This condition is pronounced in several leeward localities on the various islands—Hawaii, Maui, Molokai, Kauai—and undoubtedly obtained in Manoa.

9. THE VALLEY WALLS OR LATERAL RIDGES

The Manoa portal opens to the southwest and is bounded on east and west by the plainward terminations of its two irregularly sculptured lateral ridges. These terminal areas of the ridges may be designated as foothills; that on the east is the Manoa-Palolo foothill; that on the west is the Roundtop foothill.

The ridges extend from the coastal plain up to the main summit-ridge of the range, which here has an average elevation of 2,300 feet. Like all the ridges which define the Hawaiian valleys, these are the remnants of an original volcanic dome. The lower or foothill ends of the ridges are sufficiently bare of vegetation to reveal the laminated series of basaltic lava flows, of which they are mainly composed.

Each lateral ridge may be divided by vertical lines into three sections or areas:

1. The terminal or outlier foothill, which fronts upon and rests upon the coastal plain.
2. The transition or intermediate "knife-edge" region.
3. The mountainward region, wherein the ridge connects with or springs from the main summit ridge.

The Manoa-Palolo or East Foothill.—This, viewed from above, is a fan-shaped mass, with the expanded portion abutting upon the coastal plain. The upper slope narrows to a high (1,200 feet) apical region. The seaward slope of the foothill has an angle of about 8°; the valley wall is abrupt, rising at about 40°. The origin and physiography of the foothill is due to the remarkably localized distribution of the rainfall, as has been referred to in a previous section of the paper. The rainfall on the foothill itself is comparatively slight. Therefore erosion has advanced much more in the mountainward districts, and has left the foothill as a more or less isolated and xerophytic outlier. In the Waianae district, on Oahu, are found the culminating stages in the isolation of the foothill from the main range.

The Roundtop Foothill.—The lateral ridge which constitutes the western wall or boundary of Manoa Valley terminates in Roundtop (*Uala-kaa*). This whole ridge is distinguished by a series of ancient explosive volcanoes, of which Roundtop is the most seaward and Mount Tantalus (*Puu Ohia*) is the highest and most conspicuous. The highest points are Roundtop, 1,000 feet, Sugarloaf 1,400 feet, and Tantalus 2,013 feet. Tantalus has a well-defined crater; the craters of the other cones are either eroded away, or hidden under volcanic *ejecta*. There are a number of lesser, unnamed craters on the west ridge, and on the lower valley floor, which are very obscure, and not of phytogeographic significance.

The three named craters in prehistoric times discharged vast quantities of volcanic ashes and cinders. This material was deposited in thick blankets over the local topography, obliterating the original land features, and masked the irregularities which had been produced by erosion. Thus the surface of the Roundtop Foothill is much smoother than that of the Manoa-Palolo foothill. On the latter the surface material is the ponderous basaltic sheet lava of which the original volcanic dome was composed; on the Roundtop foothill the surface material is a secondary volcanic product—lapilli—light in weight, very porous, and produced within relatively recent geologic times.

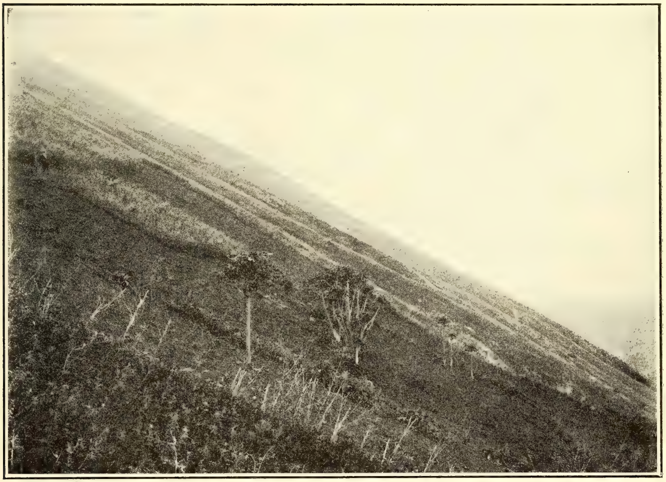

FIG. 8. View on the slopes of Round Top, showing garden patches, papaia trees and general physiography.

This difference in the nature of the surface material has resulted in a striking difference in the agricultural utilization of the two foothills. The eastern one is so rocky and rugged that it is untillable, and is used only as cattle land. The steeper slopes are covered with various introduced weeds, which have been enumerated. The Roundtop foothill, on the contrary, is rich volcanic ash, thoroughly drained, easily cultivated, and giving high yields of such crops as sweet potatoes, papaias, onions, carrots, and various other garden vegetables. There are many little garden patches on the upper slopes of Roundtop, cultivated by Portuguese, Hawaiians, Orientals, and others. The lower slopes are occupied by residences.

The outstanding ecological characteristics of the foothill region are:

1. A strong tendency toward xerophytism, indicated by the presence of many xerophytes and semi-xerophytes.
2. Brilliant insolation, due to the fact that the foothills lie seaward of the mountain cloud-cap, and under a sky which is largely cloudless.
3. Exposure to the winds, both trade and kona, owing to the smooth topography.
4. No surface water, except during and immediately after rains.
5. Topography has permitted wild live stock to overrun the foothills and to exterminate most of the native vegetation.
6. Invasion by a great variety of foreign weeds, the woody or herbaceous woody type being dominant.

10. PLANTS OF THE FOOTHILL AND ITS WALLS

Most of the plants which occur upon the foothill and its walls also occur on the valley floor; they are chiefly naturalized xerophytic ruderals.

Group I. Endemic	
Cassia Gaudichaudii Hook. & Arn.	SF
Chenopodium Sandwicheum Moq.	HSF
Lepidium Oahuense Cham. & Schl.	HPF
Lipochaeta connata DC. var. *decurrens* Hillebr.	HPSR
Nama Sandwicensis Gray	HF
Neraudia melastomaefolia Gray	SF
Phyllanthus Sandwicensis Mueller	SR
Reynoldsia Sandwicensis Gray	AR
Scaevola Gaudichaudii Hook. & Arn.	SR
Solanum aculeatissimum Jacq.	SF

Group II. Indigenous	
Andropogon contortus Roem. & Schult.	HPF
Boerhaavia diffusa L.	HSF
Chrysopogon aciculatus Trin.	HPC
Cyperus polystachys Rottb.	HPR
Daucus pusillus Michx.	HR
Dracaena aurea Mann.	AR
Euphorbia cordata Meyen	HPF
Gnaphalium luteo-album L.	HF
“ *purpureum* L.	HR
Ipomoea pentaphylla Jacq.	HVF
“ *tuberculata* Roem. & Schult.	HPVF
Osteomeles anthyllidifolia Lindl.	SVF

Panicum pruriens Trin.	HPC
" *torridum* Gaud.	HC
Phaseolus semierectus L.	HPC
Plectranthus parviflorus Willd.	HC
Plectronia odorata Benth & Hook.	SF
Pilea peploides Hook. & Arn.	HF
Sida cordifolia L.	HPC
" *rhombifolia* L.	HPC
" *fallax* Walp.	HPC
Solanum nodiflorum Jacq.	HR
Stenotaphrum Americanum Schrank	HPF
Waltheria Americana L.	HPC
Wikstroemia uva-ursi Gray	HPSR

Group III. Introduced Since the Advent of Europeans

Acacia Farnensiana Willd.	SC
Ageratum conyzoides L.	HC
Argemone Mexicana L.	HC
Asclepias Curassavica L.	HF
Bidens pilosa L.	HC
Bryophyllum calycinum L.	HPF
Cardamine hirsuta L.	HF
Cassia occidentalis L.	HPC
Cenchrus calyculatus Cav.	HPR
" *echinatus* L.	HC
Centaurea melitensis L.	HF
Cestrum diurnum L.	AR
Chenopodium album L.	HC
Clerodendron fragrans Vent.	HPVR
Crepis Japonica Benth.	HC
Crotalaria fulva Roxb.	HPC
" *saltiana* Andr.	HPC
" *spectabilis* Roth.	HPC
Cynodon Dactylon Pers.	HPF
Cyperus rotundus L.	HPC
Desmodium uncinatum DC.	HC
Eleusine Indica Gaertn.	HC
Erigeron albidus Gray	HC
" *Canadensis* L.	HC
Eugenia Jambolana L.	AR
Euxolus viridis Moq.	HF
Indigofera Anil L.	HPC
Lantana Camara L.	SC
Leucaena glauca Benth.	SF
Mirabilis Jalapa L.	HPF
Momordica charantia L.	HPVR
Nicotiana glauca Grah.	AR
Opuntia megacaniha Salm	HPC
Paspalum conjugatum Berg.	HPC
Passiflora foetida L.	HPVF
Paederia foetida L.	HPC
Phyllanthus Niruri L.	HF
Pithecolobium Saman Benth.	AF
Plantago major L.	HR
Psidium Guayava L.	SC
Prosopis juliflora L.	AC
Ricinus communis L.	SC
Salvia occidentalis Swartz.	HR
Sonchus oleraceus L.	HC
Stachys arvensis L.	HF
Stachytarpheta dichotoma Vahl.	HPC
Solanum Sodomeum L.	SF
Verbena Bonariensis L.	HPC

II. THE TRANSITION REGION

This term is used to designate the "knife-edged" portion of the lateral ridge, which lies between the foothill and the mountainward termination of the ridge in the main range. The mountainward limit of the foothill area is clearly defined by an eminence or little peak; beyond this the ridge abruptly descends and narrows. The conspicuous vertical erosion which has produced the "knife-edged" crest so characteristic of this portion of the ridge, indicates clearly the heavy rainfall to which it is subjected. The crest of the foothill is a broad, sloping, triangular plane; the crest of the transition or intermediate region is very narrow, in many places being only 2 or 3 feet in width. The valley walls of the foothill are relatively smooth and unfurrowed; the walls of the transition ridge are deeply fluted, with numerous alcoves.

The Transition Region marks the area intermediate, in ecologic features, between the high, humid ridges of the rain-forest proper, and the low, arid foothills with their covering of xerophytic and semi-xerophytic vegetation. It marks with considerable accuracy the usual seaward limit of the summit-ridge cloud-cap.

On the west ridge there is a marked discrepancy between the situations of the topographic transition region and the vegetational transition region. These two do not coincide; the topographic transition region lies two miles mountainward of the vegetational transition region. This difference is due to the presence of the Tantalus series of volcanic craters along the west ridge; these have pushed the topographic region much further mountainward than it otherwise would have occurred.

On the east ridge practically none of the normal vegetation of the lower or middle forest zones occurs seaward of the Transition Region. On the west ridge Mount Tantalus rises to a height of 2,000 feet on the seaward side of the topographic Transition Region, and supports a luxuriant lower- and middle-forest flora.

The east transition ridge is but 1,200 feet high, at its lowest point, whereas the west transition ridge is about 1,700 feet high. The rain-forest, which on the east ridge does not extend beyond the Transition Region, on the west ridge covers, not only the "transition" region, but also the mountainward half of the Tantalus mass. This condition clearly illustrates that rainfall and not topography determines the lower limits of the montane forest.

12. THE VALLEY HEAD

The head of Manoa Valley is an expanded amphitheater of erosion, rimmed by abrupt and deeply dissected walls. From the standpoint of plant life it is an ecologic complex, comprising the following elements:

1. The upper valley floor, already described.
2. A zone of broad, gentle, grassy slopes, lying above the valley floor and below the *kukui* zone. Many of these ridges are knife-edged and precipitous in their upper courses, and separate deep, narrow ravines (700–1,400-ft. contours).

3. A series of cliffs or palis, which lie between the ridges, and are more or less covered with vegetation. These cliffs are 200–300 feet high, and are cut at fairly regular intervals by V-shaped gorges and hanging valleys, from the mouths of which waterfalls issue and drop down the face of the cliffs.
4. Above the cliffs is a series of hanging valleys, separated from one another by steep ridges. These ravines have an average elevation of 1,400–2,000 feet and open above the face of the precipice. They extend abruptly back and up to the main summit ridge, a distance of .50–.75 mile.
5. The summit ridge.

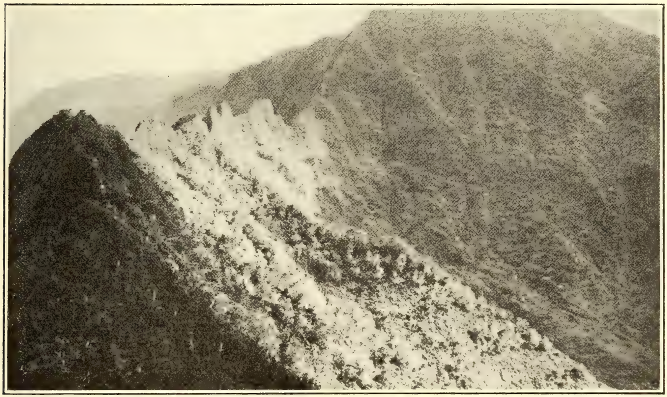

FIG. 9. Typical physiography of valley head, summit ridge and peaks. Note ravines and hanging valley formation.

The general structure of Manoa Valley, with reference to plant geography, is fundamentally the same as that of the other valleys along the leeward flanks of the Koolaus. However, variations of marked phytogeographic significance may be noted. Nuuanu Valley, for example, has cut completely through the range, and so its head is much more windswept than that of Manoa. The difference in the windiness of the heads of these two valleys has produced an observable difference in their respective vegetations, that of Nuuanu being conspicuously wind beaten. The heads of the valleys in the Punaluu region support a much finer type of forest than that of Manoa, for the former region has been practically free from the ravages of wild goats and other herbivores, and the forest is in its primitive condition. The Manoa Valley head occupies an ecologic position somewhat intermediate between the extremely arid and depleted valleys toward Makapuu Point, and the hygrophytic valleys of the central part of the range.

13. PU'U PUEO (PUPIA)

The upper floor is bifurcated by a ridge which emanates from the main summit ridge and which terminates in a green grassy hill known as Puu Pueo, the Owl Hill. This median ridge is about 2 miles long, its lowest point is 300 feet above sea-level, and Puu Pueo rises 500 feet above the valley floor. Due to the extensive erosion in the region mountainward of the hill, the ridge is conspicuously saddle-shaped, when viewed from the side.

Puu Pueo was at one time, like the region immediately adjacent to it, densely covered with the mantle of the lower forest; the ravages of wild goats and cattle, wood-cutters, and in recent times, dairy cattle, have stripped from the hill practically all of its forest growth. The principal plant now is the ubiquitous *Paspalum conjugatum;* other plants occurring here and there upon the hill are *Scaevola Chamissoniana*, *Acacia Koa*, *Microlepia strigosa*, *Cordyline terminalis*, *Clermontia macrocarpa*, *Pipturus albidus*, *Sadleria Hillebrandii*, *Osteomeles anthyllidifolia*, etc.

This ridge originally extended down the valley much further than it does at present. It is not unlikely that there were other ridges lying parallel with it, and that the physiography was considerably more complex than that of Manoa today. The present broad floor may be the result of the almost total elimination of several of these ancient ridges. Under this hypothesis the plant life of the valley under these early conditions was probably more diversified and precinctive than it is at present. Erosion has caused an infinitely gradual shifting of plant groups and zones. Projecting this vision into the future, the head of the valley will become increasingly larger, all contours more regular, and the life conditions more mesophytic. Puu Pueo will have vanished and the foothills will have been completely isolated as outliers, with low open gaps into Nuuanu and

Palolo. The foreign lowland vegetation will dominate the entire floor and its adjacent slopes.

14. THE MANOA LOWER FOREST OR KUKUI ZONE

As one views the upper portion of the valley, from the floor or mouth, the most conspicuous plant zone is the *kukui* or lower forest. This is due to the fact that the *kukui* foliage is pale silvery green, quite distinct from the yellow green of the grass lands or the heavy somber green of the rain-forest. The *kukui* groves form a broad, more or less broken band across the head of the valley.

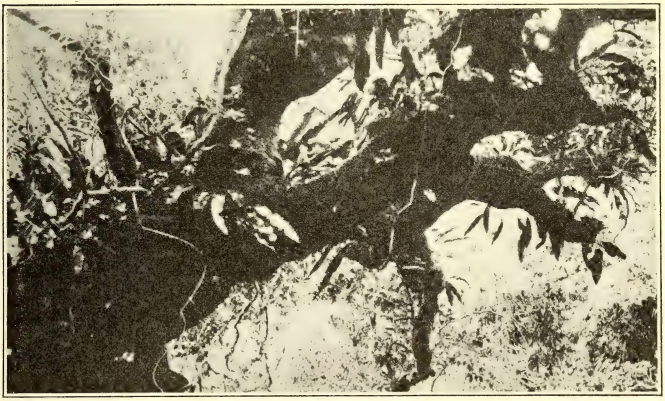

FIG. 10. *Kukui* tree in lower forest zone, covered with epiphytic plants, chiefly pteridophytes, bryophytes and lichens.

The *kukui* or Candle-nut Tree, *Aleurites Moluccana* Willd., is an euphorbiaceous tree. It was probably introduced by the primitive Hawaiians from the South Pacific, where it is abundant. It is now thoroughly established in the lower forest zone throughout the Hawaiian Islands, and is the dominant tree in many regions. It attains a height of 40–60 feet, but is usually about 30 feet high, with a broad, dome-shaped crown.

In Manoa the *kukui* occupies an irregular horizontal zone, lying across the head and around the sides of the valley, mainly between the 300- and 1,000-ft. contour lines. Along its lower fringe or level the *kukui* gives way to various species of woody or shrubby plants, conspicuous among which are: *Psidium Guayava, Lantana Camara, Osteomeles anthyllidifolia, Eugenia Malaccensis, Cordyline terminalis, Verbena Bonariensis, Hibiscus tiliaceus, Pandanus odoratissimus, Melia Azedarach,* Cassia spp., *Leucaena glauca, Bambusa,* etc. Along its upper border or level it is more or less abruptly replaced by such forms as *Acacia Koa, Metrosideros polymorpha, Ilex Sandwicensis,* Pelea spp., Pittosporum spp., *Cheirodendron Gaudichaudii,* and other rain-forest forms.

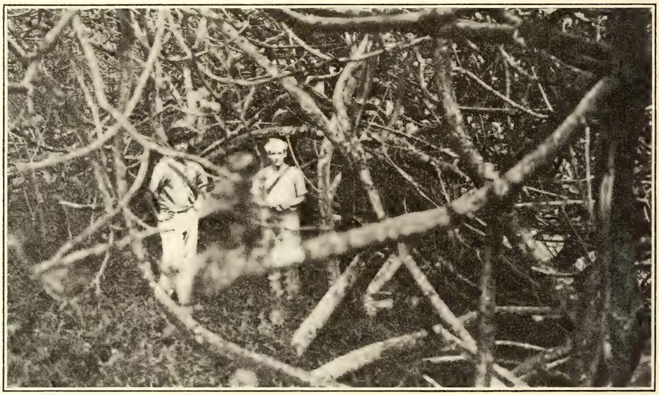

FIG. 11. In a Manoa *hau* (*Hibiscus tiliaceus*) jungle. The foliage canopy is thirty feet above the men.

Along the lateral walls of Manoa the *kukui* extends seaward until it reaches a point whereat the increasing xerophytism, and the devastations of wild goats and other pests, have inhibited its growth. There is ample evidence that in early times the *kukui* forests of Manoa extended much further seaward along the walls and floor of the valley than they do at present. There has been extensive encroachment by man and his live-stock upon all the native forests.

The *kukui* is a moisture-loving tree and in Manoa reaches its finest development in the little vales or alcoves which furrow the walls and head of the valley. It does not grow upon the exposed ridges which separate these alcoves from one another, nor upon the crests of the lateral ridges, but nestles in the alcoves. On the lateral walls it ascends to within 100 feet of the crest of the ridge. At the valley head the rain-forest rises 2,000 feet above the upper limits of the kukui zone.

The chief botanical features of the Manoa lower forest are as follows:

1. The lower forest presents a series of life conditions much less rigorous than those of the rain-forest. The slope, soil, protection from wind, and mean temperature, are all more favorable for plant development than are those same factors in the rain-forest region.

2. The lower forest, in the days of the primitive Hawaiians, was an important zone for the raising of crop plants. Twelve species were cultivated in little clearings here and there along the skirts of the lower forest. This zone was subjected to the direct and indirect effects of human utilization to a much greater degree than was the rain-forest. In ancient Hawaii the rain-forest was not much frequented by the natives—they made occasional visits for birds, canoe timbers, etc.

3. The physiognomy of the lower forest zone has been strikingly modified by introduced Hawaiian crop plants, particularly *Aleurites Moluccana*, *Cordyline terminalis*, and *Eugenia Malaccensis*. The former has become the dominant tree, and in parts of Manoa and elsewhere in the islands forms pure stands of considerable magnitude.

4. The lower boundary of this zone is undoubtedly at present at a much higher level than ever before in the history of the islands. In other words, the forested montane area is continuously diminishing; the forest margin is slowly creeping up the mountains. In geological time this movement was due to slow subsidence (according to the subsidence theory); in recent times it has been tremendously accelerated by herbivorous animals.

5. The undergrowth of herbaceous and herbaceous-perennial vegetation is much richer in the lower forest than in the rain-forest. The pteridophyte representation is much greater, however, in the latter; the Manoa rain-forest possesses 93 species, the lower forest has 40 species.

6. The lower forest tends to be more or less open, whereas the rain-forest is a completely closed association. Epiphytic vegetation is much more abundant in the rain- than in the lower forest.

15. REPRESENTATIVE PLANTS WHICH EXCLUSIVELY OR IN MOST PART INHABIT THE MANOA LOWER FOREST ZONE, KUKUI ZONE

GROUP I. ENDEMIC

Trees

Charpentiera ovata Gaud.	C
Clermontia macrocarpa Gaud.	C
Dracaena aurea Mann	R
Elaeocarpus bifidus Hook. & Arn.	C
Eugenia Sandwicensis Gray	R
Gardenia Brighamii Mann	R
“ *Remyi* Mann	R
Maba Hillebrandii Seem.	R
Ochrosia Sandwicensis Gray	R
Osmanthus Sandwicensis Knobl.	F
Perrottetia Sandwicensis Gray	F
Pipturus albidus Gray	C
Rauwolfia Sandwicensis A. DC.	C
Reynoldsia Sandwicensis Gray	F
Santalum Freycinetianum Gaud.	F
Urera Sandwicensis Wedd.	F

Shrubs and Herbaceous Perennials

Alyxia olivaeformis Gaud.	VC
Eragrostis variabilis Gaud.	C
Euphorbia Hookeri Steud.	F
“ *multiformis* Hook. & Arn.	C
Freycinetia Arnotti Gaud.	C
Gahnia Beecheyi Mann	F
“ *globosa* Mann	F
Gouldia coriacea Hillebr.	F
Joinvillea adscendens Gaud.	R
Kadua acuminata Cham. & Schlecht.	F
“ *cordata* Cham. & Schlecht.	F
Lipochaeta connata DC. var. *decurrens* Hillebr.	R
Lysimachia Hillebrandii Hook. f.	F
“ *rotundifolia* Hillebr.	R
Osteomeles anthyllidifolia Lindl.	C
Rhynchospora thyrsoidea Nees & Meyen	F
Rollandia grandifolia Hillebr.	F
“ *lanceolata* Gaud.	F
Scaevola Chamissoniana Gaud.	C
Sida Meyeniana Walp.	F
Smilax Sandwicensis Kunth	VC
Solanum Sandwicense Hook. & Arn.	F
Vaccinium penduliflorum Gaud. var. *calycinum* Hillebr.	R
Wikstroemia foetida var. *Oahuensis* Gray	F

Herbs

Anoectochilus Sandwicensis Lindl.	R
Canavalia galeata Gaud.	VR
Liparis Hawaiiensis Mann	R

Pteridophytes

Athyrium Poiretianum Presl.	F
Asplenium Macraei Hook. & Grev.	F
Cibotium Chamissoi Kaulf.	C
Doryopteris decipiens J. Sm.	C
Dryopteris nuda Underw.	F
“ *rubiformis* Robins.	F
“ *stegnogrammoides* C. Chr.	R
Polypodium Hillebrandii Hook.	R
Sadleria Hillebrandii Robins.	F
“ *polystichoides* Heller	R
Polypodium pseudogrammitis Gaud.	C

GROUP II. INDIGENOUS

Trees

Dodonaea viscosa L.	C
Maba Sandwicensis A. DC.	C
Pisonia umbellifera Seem.	C
Plectronia odorata Benth. & Hook.	F

Shrubs and Herbaceous Perennials

Adenostemma viscosum Forst.	C
Caesalpinia Bonducella Flem.	R
Dianella odorata Blume	F
Ipomoea insularis Steud.	C
Kyllingia monocephala Rottb.	F
Lythrum maritimum H. B. K.	F
Oplismenus compositus R. & Schult.	C
Panicum pruriens Trin.	C
Phytolacca brachystachys Moq.	F
Styphelia tameiameia F. Muell.	C
Zingiber Zerumbet Roscoe	C

Herbs

Commelina nudiflora L.	F
Daucus pusillus Michx.	R
Solanum nodiflorum Jacq.	C

Pteridophytes

Adiantum capillus-veneris L.	C
Asplenium caudatum Forst.	F
" *horridum* Kaulf.	C
" *lunulatum* Sw.	C
" *unilaterale* Lam.	C
Dicranopteris linearis Underw.	C
Dryopteris cyatheoides Kuntze	C
" *Sandwicensis* C. Chr.	F
Ceropteris calomelaena Link	C
" *ochracea* Robins.	C
Coniogramme fraxinea Diels.	C
Dryopteris truncata Kuntze	F
Lycopodium cernuum L.	C
Microlepia speluncae Moore	R
" *strigosa* Kaulf.	C
Neottopteris Nidus J. Sm.	C
Odontosoria Chinensis J. Sm.	C
Pellaea ternifolia Link.	F
Phymatodes elongata Presl.	C
" *Spectrum* Presl.	R
Psilotum nudum Griseb.	C
Pteridium aquilinum Kuhn	F
Pteris Cretica L.	C
Sadleria cyatheoides Kaulf.	C
Selaginella Menziesii Spring.	C
Tectaria cicutaria Robins.	C
Trichomanes Bauerianum Endl.	F
" *humile* Forst.	F
Vittaria rigida Kaulf.	C

GROUP III. INTRODUCED BY THE PRIMITIVE HAWAIIANS

Trees

Aleurites Moluccana Willd.	C
Broussonettia papyrifera Vent.	F
Eugenia Malaccensis L.	C

Shrubs and Herbaceous Perennials

Alocasia macrorrhiza Schott	C
Colocasia antiquorum Schott	F
Cordyline terminalis Kunth	C
Curcuma longa L.	R
Dioscorea pentaphylla L.	F
" *sativa* L.	C
Musa sapientum L.	C
Piper methysticum Forst.	R
Tacca pinnatifida Forst.	R
Touchardia latifolia Gaud.	F

GROUP IV. INTRODUCED SINCE THE ADVENT OF EUROPEANS

Trees, Shrubs, Herbaceous Perennials

Bambusa vulgaris Schrad. & Wendl.	C
Lantana Camara L.	C
Psidium Cattleyanum Sabine	F
" *Guayava* L.	C
Passiflora edulis Sims.	VF
" *laurifolia* L.	VF

Herbs

Bidens pilosa L.	R
Crepis Japonica Benth.	C
Physalis Peruviana L.	C
Senecio vulgaris L.	F
Sonchus oleraceus L.	F

16. ALGAE OF THE STREAMS AND WATERFALLS OF THE LOWER AND MIDDLE FOREST ZONES

Anabaena catenula Bornet.
Aphanothece Naegeli Wartmann.
Cladophora fracta Ag.
" *nitida* Kuetz.
Coleochaete irregularis Pringsh.
" *orbicularis* Pringsh.
Conferva bombycina var. *minor* Wille.
Cylindrospermum catenatum Ralfs.
" *stagnale* Bornet.
Draparnaldia macrocladia Nordst.
Fischerella ambigua Gomont.
Gloeocapsa magma Kuetz.
" *polydermatica* Kuetz.
" *quarternata* Kuetz.

Lyngbya cladophorae Tilden.
" *Martensiana* Menegh.
Mougetia capucina Ag.
Nostoc foliaceum Mougeot.
" *verrucosum* Vaucher.
Oedogonium crispum var. *Haviense* Nordst.
Oedogonium spp.
Oscillarotia sancta Kuetz.
" *formosa* Bory.
Phormidium favosum Gomont.
Phormidium papyraceum Gomont.
Schroederia setigera Lemm.
Scytonema guyanense Bornet.
" *ocellatum* Lyngb.
" *rivulare* Borzi.
" *varium* Kuetz.
Spirogyra spp.
Spirulina major Kuetz.
Stigeoclonium tenue Kuetz.
Tolypothrix distorta Kuetz.
Ulothrix minulata Kuetz.

17. RAVINES

Between the grassy ridges specified as "zone two" of the valley head are deep, narrow, steep-walled ravines, lying between the 700- and 1,400-ft. contours. These ravines are not to be confused with the hanging valleys, which occupy a higher level—1,400 to 2,000 feet—and are mantled with the true rain-forest vegetation. The ravines are occupied by plants of the *lower forest zone.* These narrow, humid gorges are the regions of minimum illumination in the valley. Their floors receive no direct sunlight until an advanced hour of the morning. The eastern arc of the sky is shut out by the mountain wall. These ravines are so narrow—their streamways are but 8 to 15 feet wide—that sunlight can enter only directly from above, and from the front, *i. e.*, facing the main valley. The subdued illumination is augmented by the cloud-cap that lies across the summit ridge. The gloominess contrasts strikingly with the glare of the main valley floor.

The larger arborescent species that are most prevalent in the ravines are: *Aleurites moluccana, Eugenia Malaccensis, Charpentiera ovata, Pipturus albidus, Urera Sandwicensis, Elaeocarpus bifidus, Clermontia macrocarpa.*

Under the shade of these trees occur a number of smaller species that are characteristically shade tolerant, for example: *Lysimachia Hillebrandii, Rollandia grandifolia, R. lanceolata, Cordyline terminalis, Smilax Sandwicensis, Oplismenus compositus, Zingiber Zerumbet, Alocasia, Colocasia, Dioscorea* spp., *Curcuma, Musa, Touchardia latifolia, Crepis japonica*, and many pteridophytes.

The plants that grow in these cool, humid, shady, protected ravines are sharply contrasted, from the ecological standpoint, with those that inhabit the hot, arid, glaring, windswept foothill slopes. These two habitats represent two environmental extremes.

18. THE ZONE OF KOA AND LEHUA

Directly above the kukui zone and commingling with it along its upper limits is the zone dominated by the *koa*, *Acacia Koa*, and the *lehua*, *Metrosideros polymorpha*. Originally the *koa* was much more abundant than it is at present; at this time practically all of the large koa has been cut or killed and the trees which remain are only of medium stature. The *lehua* is the most abundant tree in the Manoa forests, and in the forests of the archipelago as a whole. Both it and the *koa* attain their optimum development on the island of Hawaii, particularly in the region of Puna and Olaa. In these districts trees of 75–90 feet are not uncommon; in Manoa the average height is 35 feet.

The zone of *koa* and *lehua* does not have as sharp horizontal boundaries as do some of the other plant zones. The *koa* thrives in Manoa at elevations as low as 50 feet and was at one time fairly plentiful in the valley floor, in districts from which it has been absent for the last fifty years. The upper limit of the *koa* is also somewhat indefinite, averaging 1,200 feet, but sometimes rising to nearly 1,800 feet. On the island of Hawaii the finest stands of *koa* occur at elevations of 4,000 to 5,000 feet. The *lehua* occurs scattered throughout the Manoa rain-forest, particularly along the ridges, and ascends the highest peaks. On the island of Hawaii it rises to a height of 9,000 feet.

19. DISTINCTIVE FEATURES OF THE MANOA RAIN-FOREST

1. The forest flora is composed almost wholly of arborescent, shrubby, or woody species. Most of them are endemic and many are confined to the island of Oahu. There are no gymnosperms.

2. The average stature of the trees is about 25 feet; many do not exceed 20 feet. The more stunted forms occur on the steep slopes and ridge crests; along the floors of the ravines the trees may rise to heights of 30 to 40 feet.

3. Most of the shrubs are tall and semi-arborescent in character; it is difficult to discriminate between the two habits.

4. The substratum is a thin layer of stiff, red soil, derived from the basaltic lavas which directly underlie it. This soil is continuously wet, and is exceedingly tenacious of its water. It contains very little organic matter, owing to the steepness of the slope and the rapidity of the erosion.

5. The forest forms an almost unbroken mantle, covering the peaks, slopes and ravines. The only gaps are those upon the very steep cliffs, and the rents caused by landslides. The landslides vary in width from 10–40 feet and in length from 20–400 feet. At any given time there are approximately 125 landslide scars visible in the Manoa rain-forest.

6. The foliage of the rain-forest vegetation is, in general, small, simple, oval, thick, coriaceous, and with a glossy upper surface. The prevailing color is a dark, dull, heavy green, approaching olive.

7. The vegetation is very slow-growing, and relatively small shrubs and trees show that they have attained considerable age (30 to 50 years).

8. The undergrowth is scanty, and consists mainly of bryophytes and the lesser species of pteridophytes. There is practically no grass or annual vegetation.

9. The flowers of the rain-forest are small and inconspicuous. There is no well-defined flowering season, and very few showy species.

10. Tree-ferns and palms comprise a very minor element in the rain-forest. Orchids are rare. Lianas are of a relatively few species, and are not as abundant as in the lower forest zone. Plants along the summit ridges, exposed to the wind, tend to assume krumholz forms.

11. Despite the heavy precipitation, the streams of the hanging valleys and ravines of the rain-forest are exceedingly inconstant in character, filling with great rapidity after a storm, and soon running almost dry.

12. In the absence of definite records for the Manoa rain-forest, the data given by Shreve[2] for the Jamaican rain-forest may be presented as suggestive and probably very nearly the same as for Manoa:

	Temperature of the Soil	Of the Air
Annual mean	61.6° F.	60.8° F.
Annual mean range	2.9°	5.3°

The humidity of the Jamaican forest (annual summary of monthly means for 15 years), is 84.1 percent; Manoa conditions are closely comparable to this.

20. HANGING VALLEYS

Above the abrupt slopes and precipices that frame the valley head is a series of little hanging valleys. They are separated from

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[2] Loc. cit.

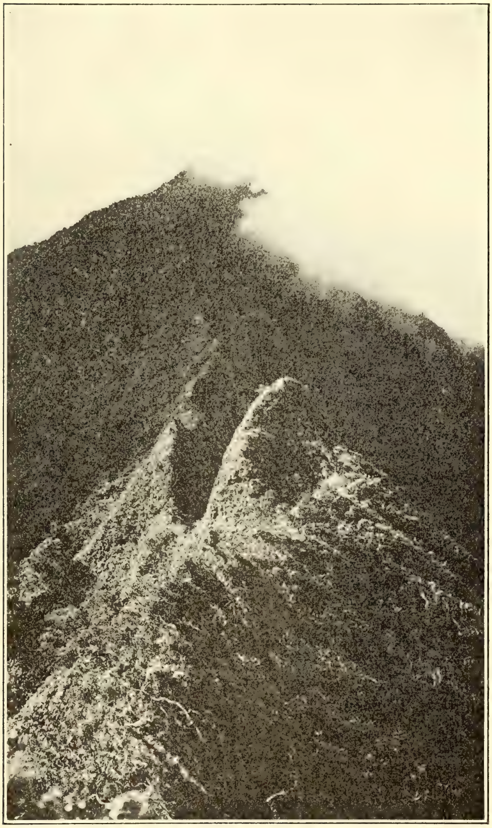

FIG. 12. Typical Koólan summit ridge and peak. Elevation of camera, about 2200 ft. Note precipices and forest mantle.

one another by steep-walled, knife-edged ridges. The ravines open upon the precipices, with vertical walls of 200 to 300 feet directly below their mouths, so they are true hanging valleys. They lie chiefly between the 1,400–2,000-ft. contours, although some reach up the slopes of Kona-hua-nui to 3,000 feet. The hanging valleys, like the summit ridges and peaks, are mantled with the somber greenery of the rain-forest.

The sides of these ravines are steep, and very difficult to climb. They are 45°–65°; the steeper declivities are constantly marked by landslides. These wounds cut through the soil to the underlying rock and remain bare for a long time.

21. SUMMIT RIDGES AND PEAKS

That portion of the main summit ridge of the Koolau Mountains which lies directly above the head of Manoa Valley, *i. e.*, between Kona-hua-nui and Olympus, is 1.7 miles long, measured along the crest. The ridge, viewed from above, is strongly curved, with its concave side facing northeast (windward), into the Ohu-ohi amphitheater. The windward wall is a great precipice, about 1,000 feet sheer, covered for the most part with scrubby vegetation, but impassable. The summit ridge forms an arc of 90°. The eastern half of this arc definitely bounds Manoa; the western half is part of the Kona-hua-nui mass. Erosion is rapidly bevelling the summit ridge, which has a strongly serrate silhuette. In the process of time a gap will be formed through the mountains, similar to the gaps at the heads of Nuuanu and Kahili Valleys. The summit ridge and peaks are covered with the dense drapery of the rain-forest.

The climate of the Manoa rain-forest is similar to that of all tropical montane forests. The temperatures are very constant and low as compared with those of the lowlands. Frost is unknown, and in the absence of accurate records, 45°–50° may be taken as a minimum. The rain-forest is far enough removed from the warm lowlands to be little influenced by them. The Oahu altitudes are not sufficient for alpine influences to be felt; this contrasts with the great mountains of the island of Hawaii, which rise to nearly 14,000 feet.

22. MOUNT KONA-HUA-NUI

Mount Kona-hua-nui is the highest peak—3,105 feet—in the Koolau Range. It lies as a mighty rampart directly northeast of the head of Manoa. Although not physiographically an integral part of the Manoa region, it is of such ecologic importance that it is considered herewith.

The airline distance from the valley-head precipices to the extreme summit of Kona-hua-nui is about one mile. The most northern branch of Manoa Stream originates at an elevation of about 2,600 feet, very near the mountain summit. There is no other point along the Manoa summit-ridge that rises above 2,400 feet, and the average is about 2,200 feet. Thus all of the Kona-hua-nui region above 2,400 feet comprises a life area which is without counterpart in any other adjacent portion of the Manoa mountains. Certain plants are very distinctive of these upper levels, and are rarely or never met below the 2,400-ft. contour. Some of these are: *Hesperomannia arborescens*, *Cheirodendron platyphyllum*, *Exocarpus brachystachys*, *Vaccinium penduliflorum* var. *gemmaceum*, *Lobelia Gaudichaudii*, *Lobelia macrostachys*, etc.

FIG. 13. Trail and camp in the Manoa rain forest. In the upper portion of a hanging valley on the side of Kona-hua-nui.

Owing to its elevation Kona-hua-nui is a great rain-maker. The trade winds become chilled in rising over it, the copious moisture condenses, and a characteristic cloud-cap covers the mountain summit during most of the year. Torrential precipitation occurs on both the windward and leeward slopes, and averages about 100 inches annually. This heavy rainfall has cut the east and west faces of the mountain into very steep precipices. The north and east faces are part of the famous Koolau *pali*. The south and west faces are fretted with hanging valleys, which debouch into Nuuanu and Manoa Valleys.

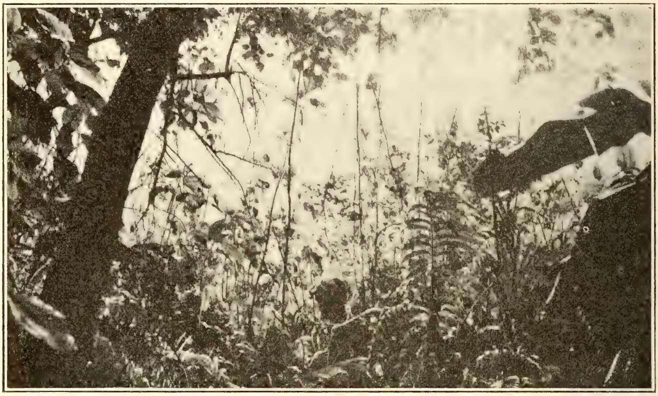

FIG. 14. View in the rain forest, showing lianas. Note man's head in center foreground, indicating height of undergrowth.

23. MOUNT OLYMPUS

Mount Olympus (Awawaloa) forms on the summit ridge the eastern boundary of the Manoa region. It rises to an elevation of 2,447 feet and closely resembles Kona-hua-nui in physiography and vegetation. It is covered with the typical rain-forest vegetation; the peak itself is wind-swept and the vegetation, like that of all the summit peaks and ridges, gives every evidence of very unfavorable life-conditions.

24. REPRESENTATIVE PLANTS OF THE MANOA RAIN-FOREST

GROUP I. ENDEMIC

Ia. Trees

Acacia Koa Gray	F
Antidesma platyphyllum Mann	F
Bobea elatior Gaud	F
Broussaissia arguta Gaud	C
Charpentiera ovata Gaud	C
Cheirodendron Gaudichaudii Seem	C
" *platyphyllum* Seem	R
Claoxylon Sandwicense Mueller	F

Dubautia plantaginea Gaud.........C
Elaeocarpus bifidus Hook. & Arn....C
Eugenia Sandwicensis Gray........R
Eurya Sandwicensis Gray..........R
Exocarpus brachystachys Hillebr.....R
Hesperomannia arborescens Gray....R
Ilex Sandwicensis Loes.............C
Labordia membranacea Mann.......C
" *sessilis* Gray.............C
" *tinifolia* Gray............F
Maba Sandwicensis DC............C
Metrosideros macropus Hook. & Arn..R
" *polymorpha* Gaud......C
" *rugosa* Gray..........C
" *tremuloides* Rock......F
Ochrosia Sandwicensis Gray........R
Osmanthus Sandwicensis Knobl......F
Pelea clusiaefolia Gray.............F
" *Sandwicensis* Gray...........C
" *rotundifolia* Gray............F
Perrottetia Sandwicensis Gray.......C
Pittosporum glabrum Hook. & Arn...F
" *glomeratum* var.
acutisepalum Hillebr..F
" *spathulatum* Mann.....F
Platydesma campanulata Mann.....F
" *cornuta* Hbd............R
Pritchardia Martii Wendl..........F
Psychotria hexandra Mann.........C
Pteralyxia macrocarpa Schult.......R
Pterotropia gymnocarpa Hillebr......R
Santalum Freycinetianum Gaud......F
Sapindus Oahuensis Hillebr.........R
Sideroxylon Sandwicense Benth. &
Hook..........................F
Straussia Fauriei Levl.............R
" *kaduana* Gray and vars....C
" *longissima* Rock..........C
" *Mariniana* Gray.........F
Suttonia Lessertiana Mez...........C
Tetraplasandra meindra Harms.....C
" *Oahuensis* Harms....F
Xanthoxylum Oahuensis Hillebr......F
" *dipetalum* Mann......R

I*b*. *Shrubs*

Sometimes more or less arborescent

Artemisia australis Less............R
Campylotheca Sandwicensis Hillebr...F
" (*Coreopsis*) *macrocarpa*
Gray, and vars......C
Clermontia macrocarpa Gaud........C
" *oblongifolia* Gaud........F
Coprosma foliosa Gray.............C
" *longifolia* Gray...........F
Cyanea angustifolia Hillebr. and
vars...................C
" *acuminata* Hillebr..........F
" *Grimesiana* Gaud...........C
Cyrtandra cordifolia Gray..........C
" *gracilis* Hillebr. and vars..R
" *grandiflora* Gaud.........F
" *Hillebrandi* Oliver.......F
" *Kalihii* Wawra...........F
" *latebrosa* Hillebr..........F
" *Lessoniana* Gray.........C
" *Macraei* Gray...........R
" *paludosa* Gaud. and vars..C
" *Pickeringii* Gray.........C
" *triflora* Gaud. and vars....F
Delissea subcordata Gaud...........C
Dubautia laxa Hook. & Arn.........F
Euphorbia clusiaefolia Hook. & Arn..C
" *Hookeri* Steud............F
" *multiformis* Hook. & Arn..C
Gouldia coriacea Gray.............C
Hibiscus Arnottianus Gray.........C
" *Kokio* Hillebr.............R
Joinvillea adscendens Gaud.........R
Kadua cordata Cham. & Schlecht....F
" *acuminata* Cham. & Schlecht..C
Labordia lophocarpa Hillebr. and
vars..........................F
Lipochaeta connata DC. var.
decurrens Hillebr.................C
Lobelia Gaudichaudii DC...........C
" *macrostachys* Hook. & Arn...F
Nothocestrum longifolium Gray......C
Pelea Lydgatei Hillebr..............R
" *oblongifolia* Gray............R
Phyllostegia glabra Benth...........C
" *grandiflora* Benth.......C
" *hirsuta* Benth..........C
" *parviflora* Benth........C
Plantago princeps Cham. & Schlecht..R
Rollandia calycina G. Don.........R

Rollandia grandiflora Hillebr ....F
“ *Humboldtiana* Gaud.......F
“ *lanceolata* Gaud. and vars..F
“ *longiflora* Wawra var. *angustifolia* Hillebr.......R
“ *racemosa* Hillebr..........R
Scaevola Chamissoniana Gaud.......C
“ *glabra* Hook. & Arn........F
“ *mollis* Hook. & Arn........F
Schiedea Nuttallii Hook...........F
Smilax Sandwicensis Kunth........VC
Solanum Sandwicense Hook. & Arn..F
Stenogyne spp...................F
Suttonia Sandwicensis Mez.........F
Tetramolopium Chamissonis Gray...R
Urera Sandwicensis Wedd..........F
Vaccinium penduliflorum Gaud......C
Viola Chamissoniana Gingins.......F
Viscum articulatum Burm. and vars..C
Wikstroemia Oahuensis Rock.......F

I*c. Herbaceous Perennials and Herbs*

Astelia veratroides Gaud...........F
Alyxia olivaeformis Gaud...........C
Baumea Meyenii Kunth...........F
Carex Oahuensis Meyer............F
Gahnia Beecheyi Mann.............F
Gunnera petaloidea Gaud...........R
Isachne distichophylla Munro.......R
“ *pallens* Hillebr.............R
Liparis Hawaiiensis Mann.........R
Peperomia blanda Kunth.
“ *Eekana* C. DC.
“ *hypoleuca* Miq.
“ *insularum* Miq.
“ *Koolauana* C. DC.
“ *latifolia* Miq.
“ *membranacea* Hook. & Arn.
“ *pachyphylla* Miq.
“ *parvula* Hillebr.
“ *reflexa* Dietr.
“ *Sandwicensis* Miq.
Rhynchospora thrysoidea Nees & Meyen........................F
Trisetum glomeratum Trin..........C

I*d. Pteridophytes*

Asplenium acuminatum Hook. & Arn.................R
“ *contiguum* Kaulf.........F
“ *glabratum* Robins........R
“ *Hillebrandii* C. Chr......R
“ *Kaulfussii* Schlecht......R
“ *lobulatum* Mett..........R
“ *nitidulum* Hillebr........R
“ *patens* Kaulf............R
“ *pavonicum* Brack........R
“ *pseudo-falcatum* Hillebr...C
“ *schizophyllum* C. Chr.....F
“ *vexans* Heller............R
Athyrium deparioides C. Chr........R
“ *proliferum* C. Chr........F
Botrychium subbifoliatum Brack.....R
Cibotium Chamissoi Kaulf..........C
“ *Menziesii* Hook..........F
“ *glaucum* Hook. & Arn......R
Cyrtomium Boydiae Robins.........R
Dicranopteris emarginata Robins.....F
Diellia pumila Brack..............R
“ *falcata* Brack...............R
Diplazium arboreum Robins.........R
“ *Fenzlianum* C. Chr.......R
Doodia Kunthiana Gaud...........C
Doryopteris decora Brack...........R
Dryopteris acutidens C. Chr.........R
“ *crinalis* C. Chr..........F
“ *Hawaiiensis* Robins......R
“ *Honolulensis* C. Chr......F
“ *Keraudreniana* C. Chr....F
“ *latifrons* Kuntze.........F
“ *nuda* Underw............F
“ *rubiginosa* Kuntze.......F
“ *squamigera* Kuntze......F
“ *unidentata* C. Chr........F
Elaphoglossum micradenium Moore..C
“ *reticulatum* Gaud.....C
Filix Douglassii Robins............R
Hymenophyllum Baldwinii Eaton....R
“ *recurvum* Gaud....C
“ *lanceolatum* Gaud..R
Lycopodium nutans Brack..........R
“ *venustulum* Gaud.......R

Lycopodium polytrichoides Kaulf.	F
Marattia Douglassii Baker	F
Odontoloma macraeanum Brack.	C
Polypodium abietinum Eaton	R
" *adenophorus* Hook. & Arn.	F
" *Haaliliolanum* Brack.	R
" *hymenophylloides* Kaulf.	R
" *pellucidum* Kaulf.	C
" *pseudogrammitis* Gaud.	C
" *pumilum* Robins.	F
" *Saffordii* Maxon	C
" *sarmentosum* Brack.	C
" *tamariscinum* Kaulf.	C
" *tripinnatifidum* Presl.	R
Pteris irregularis Kaulf.	F
Sadleria Souleytiana Moore	F
Schizaea robusta Baker	R
Schizostege Lydgatei Hillebr.	R
Selaginella arbuscula Spring.	F
" *Springii* Gaud.	F
Trichomanes cyrtotheca Hillebr.	R

GROUP II. INDIGENOUS

II*a*. *Trees*

Dodonaea viscosa L.	C
Metrosideros polymorpha Gaud.	C
Pisonia umbellifera Blume	C
Trema Amboiensis Blume	R

II*b*. *Shrubs and Herbaceous Perennials*

Dianella odorata Blume	C
Lythrum maritimum H. B. K.	F
Strongylodon lucidum Seem.	VF

II*c*. *Herbs*

Daucus pusillus Michx.	F
Nertera depressa Banks	F

II*d*. *Pteridophytes*

Adiantum capillus-veneris L.	C
Asplenium horridum Kaulf.	C
" *insiticium* Brack.	F
" *monanthes* L.	R
" *unilaterale* Lam.	C
Coniogramme fraxinea Diels.	F
Cyrtomium caryotideum Presl.	F
Dicranopteris glauca Under.	F
" *linearis* Underw.	C
Diplazium Sandwichianum Diels.	F
Dryopteris truncata Kuntze	F
Elaphoglossum aemulum Brack.	R
" *gorgonium* Brack.	F
" *hirtum* C. Chr.	C
Hymenophyllum obtusum Hook. & Arn.	R
Hypolepis punctata Mett.	R
Lycopodium cernuum L.	C
" *serratum* Thunb.	F
" *phyllanthum* Hook. & Arn.	C
Ophioglossum pendulum L.	C
Polypodium Hookeri Brack.	F
Pteridium aquilinum Kuhn	F
Pteris excelsa Gaud.	C
Psilotum complanatum Sw.	F
" *nudum* Griseb.	C
Trichomanes humile Forst.	F
" *parvulum* Poir.	F
Vittaria rigida Kaulf.	C

GROUP III. INTRODUCED BY THE PRIMITIVE HAWAIIANS

Touchardia latifolia Gaud.	F
Musa sapientum L.	C

GROUP IV. INTRODUCED SINCE THE ADVENT OF EUROPEANS

Buddleia Asiatica Lour	R
Lantana Camara L.	R
Psidium Guayava	R

SUMMARY OF THE RAIN-FOREST VEGETATION

	Species
Trees	59
Shrubs	68
Herbaceous perennials	26
Herbs	6

Endemic	198
Indigenous	37
Introduced by primitive Hawaiians	2
Introduced since advent of Europeans	3
Pteridophytes	93

Endemic Vegetation of the Rain-Forest

	Common	Frequent	Rare
Trees	18	19	13
Shrubs	26	26	12
Herbaceous-perennials and herbs	2	5	4
(11 spp. Peperomia, abundance uncertain)			
Pteridophytes	12	21	32

25. BIRDS OF THE MANOA RAIN-FOREST

All Endemic

Group I. Species that Have Become Extinct within Historic Times

Oahu Thrush, *Phaeornis Oahuensis* Wilson.
Oahu Akialoa, *Hemignathus Ellisianus* Gray.
Oahu Akiapolaau, *Heterorhynchus lucidus* Lichenst.
Oahu Akepeuie, *Loxops rufa* Bloxam; on verge of extinction.
Oahu Ou, *Psittirostra olivacea* Rothsch.
Oahu O-O, *Moho apicaulis* Gould.

Group II. Species that are Present, in Small Numbers, at the Present Time

Oahu Elepaio, *Chasiempis Gayi* Wilson.
Oahu Amakihi, *Chlorodrepanis chloris* Cabanis.
Oahu Creeper, *Oreomyza maculata* Cabanis.
Iiwi, *Vestaria coccinea* Forster.
Akakani, *Himatione sanguinea* Gmelin.

26. ORIGIN OF THE ENDEMIC FLORA

One of the most interesting problems connected with a study of the Manoa phytogeography is that of the origin of the large endemic flora, particularly that of the rain-forest. Shreve's excellent statement[3] is worthy of quotation at length:

"There is no type of vegetation in which may be found a wider diversity of life forms than exist side by side or one above the other in a tropical montane forest. Together with the structural diversities, discoverable in the field or at the microscope, are diversities of physiological behavior, discoverable by observation or experiment, and sometimes correlated with the structural features. There are quite as high degrees of specialization to be found in the rain-forest as may be sought in the desert. The prolonged occurrence of rain, fog, and high humidity at relatively low temperatures places the vegetation of a montane rain-forest under conditions which are so unfavorable as to be comparable with the conditions of many extremely arid regions. The collective physiological activities of the rain-forest are continuous but slow; those of arid regions are rapid, but confined to very brief periods. In the regions of the earth which present intermediate conditions between those of the desert and the reeking montane rain-forest may be sought the optimum conditions for the operation of all essential plant processes. It is indeed, in such intermediate regions—tropical lowlands and moist temperate regions—that the most luxuriant vegetation of the earth may be found, and it is also in such regions that the maximum origination of new plant structures and new species has taken place."

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[3] Loc. cit., pp. 109–10.

From the standpoint of conditions in the Hawaiian Islands, the closing words of the above quotation are of particular significance. Evidence is accumulating which indicates the former elevation of these islands *far above* their present levels. There undoubtedly has been a period of prolonged subsidence, amounting perhaps to several thousands of feet. The very rich endemic flora that today occupies the Manoa rain-forest very likely *did not originate there,* but rather upon warm lowlands that are now submersed beneath the ocean. In other words, Hawaii's remarkable endemic flora evolved upon prehistoric lowlands, and through slow subsidence of the land has been slowly crowded up the mountain slopes, into zones distinctly unfavorable for plant evolution. This hypothesis is also applicable to the various groups of animals—birds, snails, and insects, that today occupy the upper levels.

College of Hawaii, Honolulu

REVISION OF THE HAWAIIAN SPECIES OF THE GENUS CYRTANDRA, SECTION CYLINDROCALYCES HILLEBR.

JOSEPH F. ROCK

INTRODUCTION

The genus Cyrtandra is represented in the Hawaiian Islands by a considerable number of species. To the 32 enumerated by C. B. Clarke in his monograph, several new ones have already been added and there still remain to be described at least seven species and as many varieties.

It is to be regretted that much confusion exists in the taxonomy of the Hawaiian species. This was mainly caused through the works of C. B. Clarke and Hillebrand, both of whom described the same species of Cyrtandrae contemporaneously, the one not being aware of the other's labors.

C. B. Clarke's monograph, as far as Hawaiian species are concerned, is based mainly on the collections of Gaudichaud, Barclay, Wawra, Mann and Brigham, Nuttall, Asa Gray, and partly on specimens forwarded by Hillebrand with manuscript names.

Hillebrand had evidently not forwarded a complete set of his duplicates to Berlin and Kew, for practically none of the Hillebrand material in the Berlin Herbarium bears C. B. Clarke's determinations, whereas they are present on all specimens collected by Gaudichaud, Wawra and other earlier botanists, whose material is deposited in the various herbaria of Europe and America.

In the Berlin Herbarium, where the writer was privileged to work on the Hawaiian collection, he found C. B. Clarke and Hillebrand's species still in separate covers, notwithstanding the fact that Hillebrand's species, or at least some of them, are identical with Clarke's species.

For example, Hillebrand's *Cyrtandra latebrosa* (Fl. Haw. Isl. 337. 1888) is *Cyrtandra longifolia* Hillebr. var. *degenerans* C. B. Clarke, and published as such in the latter's monograph on the tribe *Cyrtandreae.* Hillebrand distributed material of this species under *Cyrtandra longifolia*, which name was adopted by Clarke, giving Hillebrand credit for it as author, while Hillebrand himself published the same species under the name *Cyrtandra latebrosa* without mentioning his former manuscript name. On still another sheet we find for the same species still another name—*Cyrtandra paradoxa*. Again, a specimen in the herbarium at Vienna, No. 1991, marked *Cyrtandra paludosa* Gaud. var. *α longifolia* Wawra, which is a synonym of *Cyrtandra longifolia* Hillebr. (in Clarke Monogr. 276. 1883), was selected by C. B. Clarke as the type for Hillebrand's manuscript name *C. longifolia*.

It is the writer's desire to clear up all this confusion. He was in a position to examine the material which formed the basis for C. B. Clarke's monograph, and he also compared the same with Hillebrand's collection.

The larger portion of Hillebrand's material was not known to C. B. Clarke, as it came into the possession of the Berlin Herbarium after Hillebrand's death.

This paper is the first of a series on the genus Cyrtandra and contains a critical revision of the section Cylindrocalyces Hillebr.

SECTION ONE: CYLINDROCALYCES Hillebr. Fl. Haw. Isl. 326. 1888

Calyx campanulate, cylindrical or fusiform, five-cleft into unequal lobes, splitting laterally at last; corolla usually large, curved, bilabiate; flowers single or few, subumbellate to cymose; leaves mostly glabrous, or, when tomentose, usually so along the midrib and nerves underneath, with blackish or dark brown to reddish hair, often thickly matted (*C. longifolia*, var. *degenerans*).

This section possesses now seven species, ten varieties, and four forms, of which one species, two varieties, and four forms, are here described for the first time.

The species are closely related, for example: *C. paludosa* and *C. longifolia; C. cyaneoides* and *C. waianuensis; C. grandiflora* and *C. filipes; C. oenobarba* var. *petiolaris* appears to come more or less close to *C. longifolia* var. *calpidicarpa*, in the long cylindrical fruit.

CYRTANDRA PALUDOSA Gaud. Bot. Voy. Uranie 447. 1830. Var. *α* TYPICA C. B. Clarke Monogr. Cyrt. 5: 275. 1883–1887

A low shrub, the young parts silky to rusty-tomentose; branches glabrous, somewhat fleshy; leaves opposite, elliptical-oblong, thick, chartaceous, acuminate at both ends, with crenate to serrate margins, almost glabrous when mature, dark green above, pale underneath, the veins straight and prominent, 10–22 cm. long, 4–6 cm. wide, on petioles of 2–6 cm.; peduncle short, 5 mm. long; cyme few-(3–7) flowered; bracts 6–8 mm., long-acuminate, covered with reddish brown hair; pedicels 6–10 mm. long; calyx cylindrical to campanulate, thin, 10–15 mm., unevenly 5-fid to the middle or less into lanceolate, acuminate lobes, splitting laterally, caducous when with fruit, partly hirtellous or glabrous; corolla 15–20 mm., suberect, exserted, glabrous, white; fruit 12–20 mm. long, 5–8 mm. broad, glabrous.

Oahu: ex. Coll. Gaudichaud no. 154, Iles Sandwich, visit 1841, in herb. Berlin, and part of type in College of Hawaii herbarium; Ins. Sandwic. Oahu, Meyen 5/31, labeled *C. Garnottiana* det. C. B. Cl. *C. paludosa*, and Meyen *C. triflora* Gaud. det. C. B. Cl. *C. paludosa* Woahoo, Ins. Sandw. Macrae, Maio 1825, in herb. Soc. Hort. Lond. and in herb. Berlin; Lindley visit 1832 in herb. Berlin; Hawaiische Inseln, Wawra no. 1665, Oahu, fruiting and flowering (three sheets) in herb. Vienna and herb. College of Hawaii, and no. 2375 leg. Hbd. comm. Dr. Wawra, in herb. Vienna; Niu Valley, Oahu, leg. Lydgate, Willi, 1870, herb. Hillebr. Berlin; Kalihi, Oahu, Jan. 1870, leg. Hbd. fruiting specimen in herb. Berlin; Palolo Valley, main ridge, flowering, Nov. 7, 1908, Rock no. 96 in herb. College of Hawaii; Punaluu Mts., Koolau, flowering Nov. 14–21, 1908, Rock no. 291 in herb. College of Hawaii; Waikane Mts., flowering, Jan. 23, 1909, Rock no. 1251 in herb. College of Hawaii.

The Oahu specimens are the typical *C. paludosa α typica* C. B. Clarke. The species occurs on Hawaii also, but is much smaller in every way.

Hawaii: Kilauea, leg. Hillebr. April 1868, flowering, in herb. Berlin; Hilo, leg. Lydgate in herb. Berlin (with small narrow leaves); Kalanilehua, Kilauea, flowering, May 1912, Rock no. 10343 in herb. College of Hawaii; Alakahi Kawainui along ditch trail, flowering and fruiting, July 13, 1909, Rock no. 4473 (two sheets) in herb. College of Hawaii; Alakahi ditch in swampy forest, flowering, June 1910, Rock no. 8513 in herb. College of Hawaii.

The specimens from Alakahi and Kawainui gorges, near the summit of the Kohala mountains, at an elevation of 4,200–4,500 feet, differ considerably from the typical specimens occurring on Oahu; on Hawaii where they grow in dense swampy forests in thick Sphagnum moss they are only 2–3 feet in height, the leaves are smaller, ovate-elliptical, much more coarsely serrate, of thicker texture, and on shorter petioles; the peduncles are shorter than in the Oahu specimen, or are almost wanting; the calyx is glabrous and not thin. It would perhaps be better to class these plants as a distinct variety, but owing to the polymorphism of most of the Hawaiian plants it may cause confusion if raised to varietal rank.

The plants from Kilauea (no. 10343) have still smaller leaves (elliptical-lanceolate) and the branches are very slender, otherwise the same.

Cyrtandra paludosa Gaud. var. brevicalyx Hillebr. Fl. Haw. Isl. 336. 1888

Leaves as in the species *α* typica C. B. Cl. though broader, on stout petioles of 2.5–5 cm.; peduncle very short or almost wanting, often only one-flowered, the filiform pedicels 18–36 mm.; calyx only one third the length of the corolla, 6–8 mm., cyatiform with broad acuminate lobes or teeth; berry slender, fusiform, 20–24 mm. long.

Oahu: Kaala range, Hillebrand, July 1870, in Herb. Berlin (doubtfully referred here a specimen from the Waikane Mts. flowering and fruiting Jan. 23, 1909, Rock no. 1135 in herb. College of Hawaii).

This variety differs from the species in the slender, long pedicels, short peduncle and small calyx, perhaps only a form of the typical species.

The writer's specimen from the Waikane Mts. have long, very slender, pedicels (a little over 18 mm.) but a distinct peduncle, the calycine lobes being very short as is the calyx tube.

Cyrtandra paludosa Gaud. var. alnifolia Hillebr. Fl. Haw. Isl. 336. 1888

Young shoots and inflorescence hirsute with dark ferruginous hairs; leaves broadly ovate, 10–12.5 cm. long, 6–7.5 cm. wide, somewhat obtuse, rounded at the base, the strong ribs and veins pubescent; peduncle and pedicels 12–14 mm. each; calyx and corolla faintly pubescent.

Oahu: Hillebrand in herb. Berlin.

This variety can be retained; in its general appearance it is a typical *C. paludosa* but differs from *α typica* only in the longer peduncles and pedicels, and in the young leaves, which are covered with a fulvous tomentum.

Cyrtandra paludosa Gaud. var. microcarpa Wawra Flora **55**: 560. 1872 (not Hillebr.)

Plant low, 1 m., rarely more, scarcely branching; leaves as in *C. paludosa α typica* though somewhat larger, light green above, fawn colored underneath, the midrib and veins prominent, covered with a silky, brown pubescence; petioles about 4 cm.; inflorescence densely clustered in the leaf-axiles covered with a brown, coarse pubescence; peduncles short, 0–4 mm., many-flowered; pedicels 5–20 mm., umbellate; calyx 8 mm. long, subglabrate, caducous; corolla 12–14 mm. long, tube narrow, curved, glabrous; fruit 1 cm. long, 5–6 mm. broad, numerous, subconglomerate.

KAUAI: In the forests above Waimea, Wawra no. 2056 in herb. Vienna, and herb. College of Hawaii; Kealia and Waimea leg. Knudsen, in herb. Berlin; at the head of Olokele canyon along rockwalls, flowering Oct. 1909, Rock no. 5414 in herb. College of Hawaii.

Hillebrand's var. *δ microcarpa* is identical with Wawra's var. *microcarpa* and is therefore preoccupied by Wawra; there are three sheets in the Vienna herbarium ex Coll. Wawra.

Hillebrand says: "including probably Wawra's var. *confertiflora* and *herbacea*." His variety *confertiflora* does not belong to *C. paludosa* Gaud.; it was described by C. B. Clarke in his monograph as *C. confertiflora* (Wawra) Clarke and that justly, for the plant has no resemblance to *C. paludosa.*

Wawra's var. *herbacea* does not belong to *C. paludosa* Gaud. but to *C. oenobarba* and Heller's combination (*C. oenobarba herbacea* Heller) is therefore correct.

CYRTANDRA PALUDOSA Gaud. var. SUBHERBACEA Wawra, Flora **55**: 559. 1872

Plant glabrate; leaves broadly ovate or suborbicular, rounded at the base, shortly acuminate at the apex, on long, stiff petioles; inflorescence almost as in *C. paludosa typica;* peduncle glabrous, but with a reddish tomentum at the base, 3–7-flowered; calyx subcampanulate, membranaceous, glabrous, caducous; lobes little smaller than in *C. paludosa typica*, and subdeltoid.

KAUAI: plateau of Waialeale, Wawra no. 2155 in Herb. Vienna (two sheets), det. by C. B. Clarke; part of type in herb. College of Hawaii.

CYRTANDRA PALUDOSA Gaud. var. **Gayana** (Heller) Rock.

Cyrtandra Gayana Heller, Minn. Bot. Stud. **9**: 887, pl. 59. 1897.

A small tree, 3 m. high, the trunk usually 10 cm. in diameter; leaves opposite, lanceolate, tapering at both ends, 5–7.5 cm. long, about 2 cm. wide, entire, bright green above, with impressed midrib and veins, covered with a brown pubescence underneath, the petioles

1.5 cm.; flowers solitary or two, in the axils of the leaves, on a peduncle of 3 mm.; pedicels 6–15 mm.; calyx thin, slightly pubescent, as in *C. paludosa typica;* fruit ovate-oblong, 10 mm., tipped with the persistent style.

KAUAI: on the ridge west of the Hanapepe river, elevation 3,000 feet, and on the plateau above Waimea 4,000 ft. elevation, Heller, no. 2495; forests of Kaholuamano, above Waimea, flowering, March 3–10, 1909, Rock no. 2280 in herb. College of Hawaii; flowering and fruiting Sept. 1909, Rock, no. 5600 in herb. College of Hawaii.

Heller says: "It belongs to the group of which *C. paludosa* is the type."

In fact it is merely a variety of *C. paludosa*, differing from it in its smaller, entire leaves and arborescent habit, otherwise the same. Heller states that it may be identical with Wawra's *Cyrtandra paludosa* var. *arborescens.* This is however not the case. The writer was able to examine Wawra's plant, through the courtesy of Dr. Alexander Zahlbruckner of Vienna. Wawra's plant is now *C. longifolia* Hillebr. var. *arborescens* C. B. Clarke, and is identical with Hillebrand's *C. paludosa* var. *integrifolia.*

CYRTANDRA PALUDOSA Gaud. var. **haupuensis** Rock n. var.

A small bush with thick angular branches; young shoots pubescent; leaves opposite, elliptical-olbong, subcoriaceous, dark green above, light brown underneath, glabrous on both sides, subentire, with a slightly undulate margin and faint crenation, 15–22 cm. long, 3.5–5 cm. wide, acute at the apex, gradually narrowing at the base into broadly auriculate margins; petiole 1 cm. long; inflorescence axillary; peduncle 1–2 mm., 3–4-flowered; pedicels 10–12 mm.; calyx thin, glabrous, caducous, nearly as long as the tube of the corolla; fruits (immature) cylindrical-oblong, acuminate at the apex.

KAUAI: Lihue, near the summit of the Haupu range, flowering and fruiting March 18, 1909, Rock, type no. 2473 in herb. College of Hawaii.

A very distinct variety, nearly worthy of specific rank; it differs from the species in its robust habit, subcoriaceous, subentire, auriculate leaves, and very short-peduncled inflorescence.

SPECIMINA EXCLUDENDA

C. PALUDOSA Gaud. var. HERBACEA Wawra no. 2070 in herb. C. B. Clarke = *Cyrtandra oenobarba* Wawra var. *herbacea* Heller.

C. PALUDOSA Gaud. var. CONFERTIFLORA Wawra no. 2057 in herb. Vienna = *Cyrtandra confertiflora* C. B. Clarke.

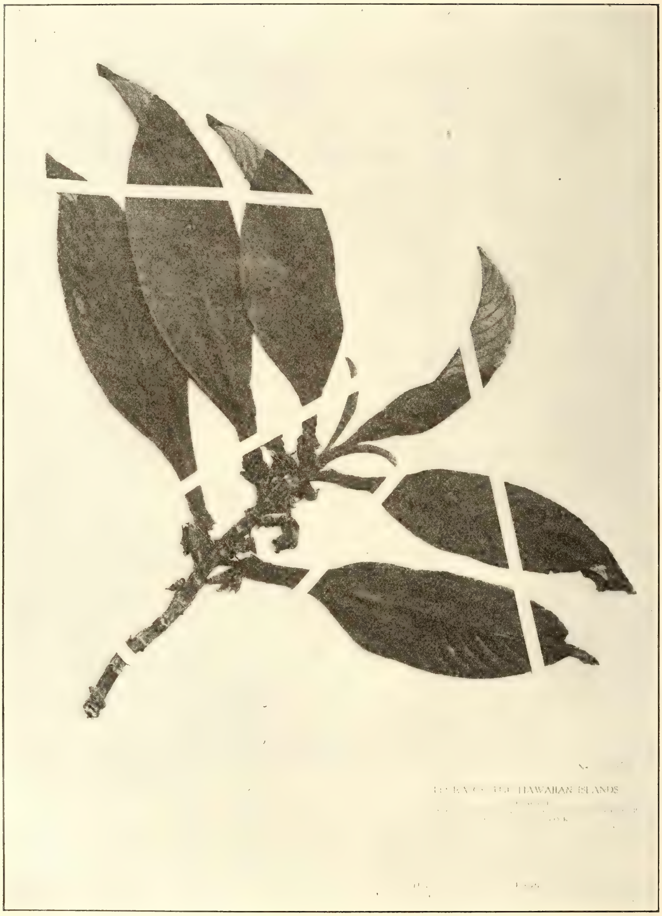
HAWAIIAN ISLANDS

FIG. 1. *Cyrtandra paludosa* Gaud. var. *haupuensis* Rock. Type in the College of Hawaii Herbarium.

C. PALUDOSA Gaud. var. INTEGRIFOLIA Hillebr. Knudsen no. 137, Kauai, in herb. Berlin = *Cyrtandra longifolia* Hillebr. var. *arborescens* C. B. Clarke.

Hillebrand's specimen from the Kohala Mts. Hawaii and referred by him to the above variety with Knudsen's no. 137, is an entirely different plant and has absolutely nothing in common with *C paludosa* or with *C. longifolia;* the leaves remind one very much of a species of *Shorea*. It represents an undescribed species.

CYRTANDRA LONGIFOLIA Hillebr.; C. B. Clarke, Monogr. Cyrt. 5: 276. 1883–87

Cyrtandra paludosa Gaud. var. *longifolia* Wawra, Flora 55: 558. 1872.

Branches scarcely quadrangular, glabrate, the young parts hirsute with ferruginous hair; leaves opposite, shortly petiolate, 0–1 cm., elongate-oblong, acute, subentire, 22 cm. long, 5 cm. wide, nearly glabrous when mature, with a yellowish wool along the median nerve on the lower surface; peduncles 0–5 mm., often one-flowered; bracts narrow; pedicels 3 cm. long, or longer, with a reddish-brown tomentum; calyx 12 mm., the tube campanulate, with a reddish-brown tomentum outside, the lobes deltoid-acuminate; tube of corolla 14 mm., cylindrical, curved upwards, glabrous outside, the lobes 7 mm. long, 4 mm. wide, minutely pubescent inside; fruit 22 mm. long, 1 cm. broad, broadly-oblong; calyx at first ampliate, persistent, later caducous.

KAUAI: Hanalei forests, collected by Wawra flowering and fruiting, no. 1991 in herb. Vienna.

This species is only known to the writer from the type which is Wawra's No. 1991 in the herb. Vienna.

There are two sheets in the Vienna herbarium both bearing the number 1991.

The flowers are on long pubescent pedicels.

CYRTANDRA LONGIFOLIA Hillebr. var. ARBORESCENS C. B. Clarke, Monogr. Cyrt. 5: 276. 1883–87

Cyrtandra paludosa Gaud. var. *arborescens* Wawra, Flora 55: 558. 1872.

Cyrtandra paludosa Gaud. var. *integrifolia* Hillebr. Fl. Haw. Isl. 337. 1888.

Branches thick, woody; leaves subentire, lanceolate, broader towards the apex, as in *C. longifolia typica*, but attenuate at the base

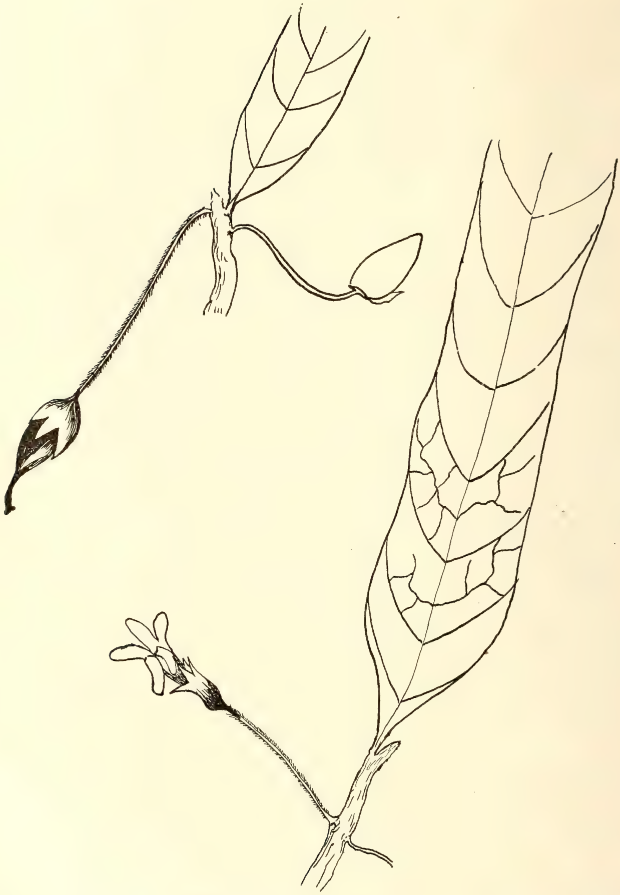

FIG. 2. *Cyrtandra longifolia* Hillebr. Sketched from the type in herb. Vienna, ex coll. Wawra, no. 1991. Hanalei, Kauai.

and merging into a winged petiole of 2–7 cm., or subsessile; peduncels nearly wanting, one-flowered, the rigid pedicels 1–2 cm., with reddish tomentum; calyx 16 mm. long, glabrous, caducous; fruit ovoid-elliptical.

KAUAI: Wawra no. 1991 *b* (not *c*, as given by C. B. Clarke) in herb. Berlin, and portion of type in herb. College of Hawaii.

There are two sheets of this variety in the Vienna Herbarium; not represented in the Berlin Herbarium. This variety is only known to the writer from Wawra's collection.

Hillebrand's *C. paludosa* var. *integrifolia* from Kauai belongs here, rather than to the species on account of the much shorter pedicels.

CYRTANDRA LONGIFOLIA Hillebr. var. DEGENERANS C. B. Clarke
Monogr. Cyrt. 5: 277. 1883–87

Cyrtandra paludosa Gaud. var. *degenerans* Wawra, **55**: Flora 558. 1872.

Cyrtandra paradoxa Hillebr. ms.

Cyrtandra latebrosa Hillebr. Fl. Haw. Isl. 337. 1888.

Cyrtandra Hawaiiensis Drake del Cast. Ill. Fl. Ins. Mar. Pacif. **7**: 253. 1892, not C. B. Clarke.

Cyrtandra degenerans (Wawra) Heller, Minn. Bot. Stud. **9**: 887. 1897.

Stem straight, 2–4 m. high, with a thick glutinous sap, the young shoots and inflorescence dark ferruginous, with a thick squamaceous tomentum; leaves verticillate, 3–5 in a whorl, narrow-oblanceolate, 12–25 cm. long, 2–5.5 cm. wide, acute to acuminate, entire or shortly dentate, chartaceous, dark green above, brownish underneath with a short and soft tomentum, prominently penninerved; peduncle very short, 2–4 mm., 1–5-flowered, the flowers drooping on pedicels scarcely longer than the peduncle; bracts linear-lanceolate, 10–14 mm. long; calyx caducous, fleshy, shaggy outside and inside with dark squamaceous tomentum, 20–30 mm. long, fusiform in the bud, with a lateral slit through which the corolla protrudes, the peaked top remaining entire or splitting into five short teeth; corolla slightly exserted, glabrate, curved, with large spreading limb, bilabiate, the upper lip deeply emarginate, the lower three-lobed, 8–10 mm. long; style twice as long as the glabrous ovary; berry olive-shaped, 26 mm. long.

OAHU: Wawra no. 1781 (two sheets) in herb. Vienna (type), and part of type in herb. College of Hawaii; in deep and dark ravines of Kalihi and Manoa, Hbd. without date or number, in herb. Berlin and herb. College of Hawaii (Kalihi spec.); Mts. of Punaluu, Koolau range along stream bed, flowering Aug. 1908, Rock, no. 9 in herb. College of Hawaii; Punaluu Mts., flowering Nov. 14–21, 1908, Rock

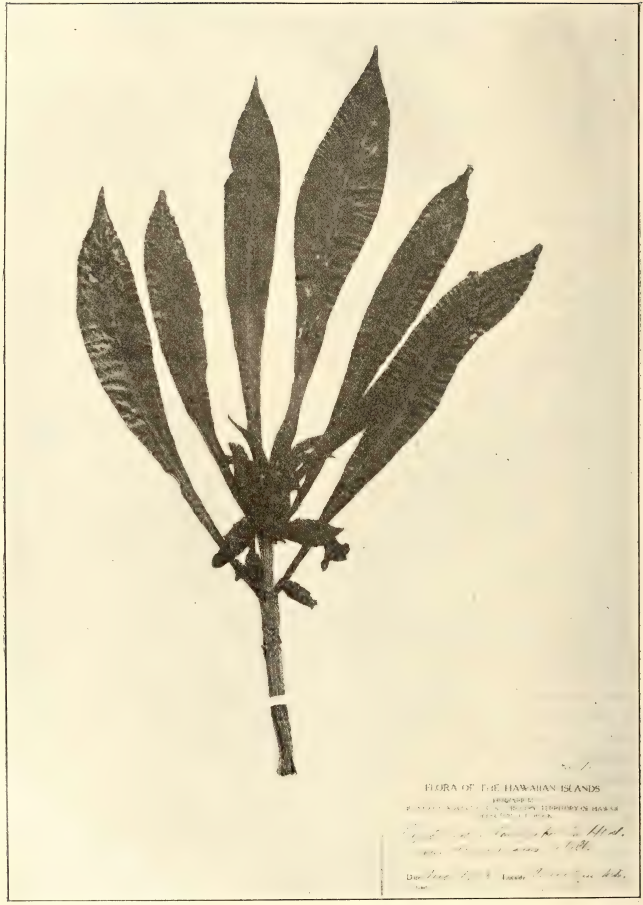
FLORA OF THE HAWAIIAN ISLANDS
Date
Locality

FIG. 3. *Cyrtandra longifolia* Hillebr. var. *degenerans* C. B. Clarke. [Typical specimen.

no. 933, 934; flowering Dec. 3–14, 24–29, 1908, Rock no. 157 & 407 in herb. College of Hawaii; Kaukonahua gulch, Wahiawa, flowering, May 15, 1909, Rock, no. 3029 in herb. College of Hawaii.

MOLOKAI: Mapulehu Valley, Hbd. in herb. Berlin without date or number.

The Oahu specimens are the typical var. *degenerans;* the plant occurs in the very wet forests usually in deep ravines and along stream beds in dense shade. It usually does not branch, but bears a crown of leaves at the end of a stem 3–4 m. high, with the inflorescence clustered in the leaf-axils. It is a rather handsome plant and resembles somewhat certain species of the lobelioideous genus *Cyanea* in habit.

Hillebrand's specimens from Kalihi are identical with the writer's plants from the Punaluu Mts. of the same range.

CYRTANDRA LONGIFOLIA Hillebr. var. DEGENERANS C. B. Clarke, forma **subglabra** Rock

Cyrtandra latebrosa Hillebr. var. *β subglabra* Hillebr. Fl. Haw. Isl. 338. 1888.

Leaves quaternate, almost glabrate underneath, only the midrib and veins reddish-tomentose, pale on both sides, shortly dentate, thin, chartaceous, obovate-oblong, acute to acuminate, 16–30 cm. long, 4–6.5 cm. wide, gradually contracting toward the base, subsessile or running out into a broadly winged petiole; calyx membranous, hirsute with dark brown hair, occasionally glabrate outside, but hirsute inside, fusiform; corolla as long as the calyx, only the lobes exserted, slightly hairy or glabrous.

MOLOKAI: Kalae, Hillebrand in herb. Berlin without date or number; Mapulehu Valley, flowering March 1910, Rock no. 12518 in herb. College of Hawaii.

MAUI: Honomanu Valley, along stream bed, northern slope of Mt. Haleakala, flowering, May 1911, Rock no. 12519.

HAWAII: Valley of Holopalau in Kohala, Hbd.; Hamakua, Paauhau no. 3, forest, flowering July 5, 1909, Rock nos. 4061, and 4062; Holokaiea gulch, back of Waimea, flowering and fruiting July 10, 1909, Rock no. 4081 in herb. College of Hawaii.

The Maui and Hawaii specimens have green, thin, glabrous calyces, and only the pedicel and nervature of the calyx is slightly hirsute, while the Molokai specimens have the whole calyx densely hirsute.

The leaves in the Maui and Hawaii specimens are also thinner and broader towards the apex, than those of Molokai.

The specimen which Hillebrand records from Waiehu, Maui, belongs to the writer's forma *cymosa.*

CYRTANDRA LONGIFOLIA Hillebr. var. DEGENERANS Wawra, forma **cymosa** Rock n. f.

Branches angular; leaves quaternate, broadly obovate-oblong, thin chartaceous, sparingly pubescent on both sides, dark green above, paler underneath, irregularly dentate, acute at the apex, 14–24 cm. long, 4.5–9 cm. wide, contracting at the base into a broadly margined petiole, subsessile, or on petioles of 2–3 cm.; inflorescence a cyme, hirsute with brownish hair throughout; bracts linear-lanceolate, acute, to subfoliaceous; peduncle 1.5–3 cm., 3–8-flowered; pedicels 1.5–2.5 cm.; calyx not fusiform, split into subdeltoid or linear-lanceolate, acute lobes, nearly glabrate or hirsute with brownish hair; corolla exserted; berry unknown.

MOLOKAI: Mapulehu Valley, flowering March 1910, Rock no. 10334 in herb. College of Hawaii.

MAUI: Valley of Waiehu, Hillebrand, without date or number.

HAWAII: Holokaiea gulch back of Waimea, elevation 4,000 ft., flowering July 10, 1909, Rock nos. 4075 and 4479 in herb. College of Hawaii.

The Hawaii specimens differ slightly from the Molokai specimens in the larger and denser flowered cyme and the linear-lanceolate, acute calycine lobes.

CYRTANDRA LONGIFOLIA Hillebr. var. DEGENERANS C. B. Clarke, forma **oppositifolia** Rock n. f.

Branches quasi quadrangular; leaves opposite, obovate-oblong, acute at the apex, hirtellous on both surfaces, especially along the prominent midrib and nerves, with brownish hair, 15–22 cm. long, 4–6 cm. wide, gradually contracting into a petiole of 2–3 cm.; flowers single, or three on a common peduncle of 1–1.5 cm.; bracts linear-oblong, acute, 12 mm.; pedicels 8–10 mm.; calyx not fusiform, split to near the base into 5, linear-oblong, acuminate lobes; peduncle, pedicels, and calyx hirsute with reddish-brown hair; corolla slightly exserted, lobes large, spreading, of unequal size, hirtellous or nearly glabrate; berry unknown.

MAUI: Western division, Honokawaii gulch, flowering Aug. 1910, Rock no. 8206 in herb. College of Hawaii.

Differs from the other forms in the opposite leaves, deeply divided calyx, and large spreading corolla lobes.

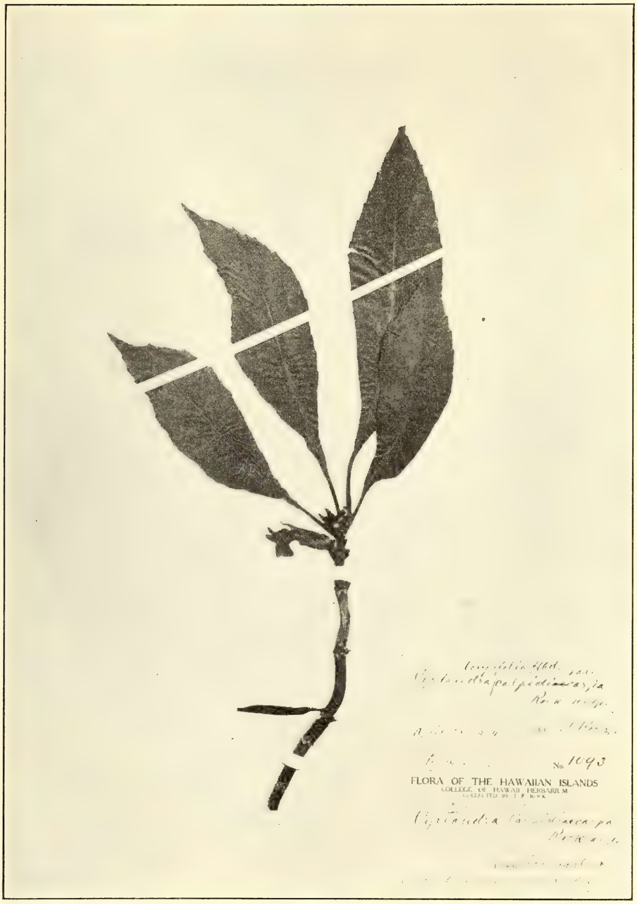
No. 1093
FLORA OF THE HAWAIIAN ISLANDS
COLLEGE OF HAWAII HERBARIUM
COLLECTED BY J. F. ROCK

FIG. 4. *Crytandra longifolia* Hillebr. var. *calpidicarpa* Rock. Type in the College of Hawaii Herbarium.

CYRTANDRA LONGIFOLIA Hillebr. var. DEGENERANS C. B. Clarke, forma **auriculaefolia** Rock n. f.

Stem fleshy; leaves quaternate, broadly obovate-oblong, obtuse at the apex, dark on both surfaces, as well as slightly pubescent, 18 cm. long, 5.5–6.5 cm. wide, subsessile, and broadly auriculate, with a basal diameter of about 3 cm. in the older leaves; peduncle 3 mm., usually three-flowered; pedicels 5 mm.; calyx fusiform, thin, 20 mm. long, subglabrous; corolla slightly exserted, the lobes small, unequal.

MAUI: Western division, Honokawai gulch, deep, shaded places, along the stream, flowering Aug. 25, 1910, Rock no. 8159 in herb. College of Hawaii.

Differs from the other forms in the dark, broadly obovate-oblong, auriculate leaves.

CYRTANDRA LONGIFOLIA Hillebr. var. **calpidicarpa** Rock n. var.

Shrub 1 m. high, branching; leaves quaternate, elliptical-oblong, membranous, pale underneath, light green above, glabrous on both sides, excepting a reddish brown pubescence along the midrib, shortly and unevenly dentate, subentire in the lower portion, acuminate at the apex and base, 10–16 cm. long, 3–4 cm. wide, on a petiole of 2.5–3 cm.; peduncle 2 mm., 2–3-flowered, reddish-tomentose; pedicels 2 mm.; bracts foliaceous nearly as long as the calyx, the latter caducous, thin, fusiform, glabrate, excepting the acuminate lobes; corolla curved, exserted, 20 mm. long, including the spreading, subequal lobes; berry long-cylindrical, 3.5–4 cm. long, 4 mm. wide.

OAHU: Windward side, Waiahole Valley, on rocky wall, near waterfall at the head of the valley; flowering and fruiting Jan. 17, 1909, Rock, type no. 1093 in herb. College of Hawaii.

Remarkable for its long cylindrical fruit which, in shape, reminds one of those of Calpidia. It is so far the only Cyrtandra found in these Islands, with a 4 cm. long, cylindrical fruit.

Cyrtandra Waianuensis Rock n. sp.

Plant 1.5–2 m. high, erect, single stemmed, not branching; stem somewhat fleshy towards the apex, thick, woody and brittle towards the base, with a large crown of sessile leaves at the apex; leaves broadly oblong, subentire, or faintly dentate, dark green above, pale underneath, glabrous above, pubescent below, with fine yellowish-brown hair, obtuse or subacute at the apex, 30–45 cm. or more long, 15–20 cm. wide, thin, membranous to chartaceous, suddenly contracting at the base, sessile to subsessile; inflorescence densely clustered in the axils of the leaves on a common peduncle of 2–3 mm., with

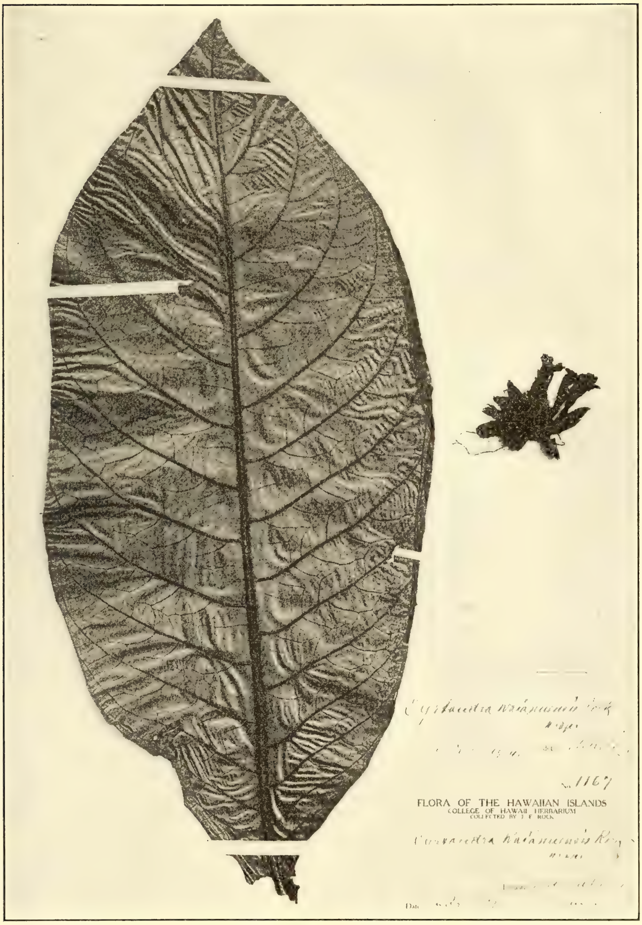
Cyrtandra Waianuensis Rock
No. 1167
FLORA OF THE HAWAIIAN ISLANDS
COLLEGE OF HAWAII HERBARIUM
COLLECTED BY J. F. ROCK
Cyrtandra Waianuensis Rock

FIG. 5. *Cyrtandra Waianuensis* Rock. Type in the College of Hawaii Herbarium.

numerous bracts at the apex, bracts oblong, acute, 12 x 4 mm.; pedicels 1 cm.; calyx caducous, thin, glabrous, green, fusiform, 15 mm. long, the lobes acute, short; corolla slightly exserted, puberulous, the lobes very small and subequal; berry (immature) ovate-oblong, 14 x 5 mm.

OAHU: Waianu Valley, windward side of the Koolau range, near the head of the valley, along stream-bed, flowering, Jan. 22, 1909, Rock, type no. 1167 in herb. College of Hawaii.

A very interesting and striking species, remarkable for its large leaves, which are sessile. The species is single stemmed and at first glance resembles some of the Hawaiian Lobeliads, especially species of the genus Rollandia, in whose company the plant grows.

It is related to *Cyrtandra longifolia* var. *degenerans* and its forms.

CYRTANDRA CYANEOIDES Rock, Bull. Coll. Haw. **2**: 39. 1913

Plant subherbaceous, somewhat woody at the base, the stem erect, not branching, 11–12 dm. high, 4 cm. in diam., bearing a crown of leaves at the apex, not unlike a species of Cyanea; leaves 45–55 cm. long, including the thick, winged petiole, 22.5–35 cm. broad; midrib fleshy, obovate, rounded at the apex, suddenly narrowing below into a margined petiole, the latter 1.5 cm. thick, texture of leaves thick, but coriaceous to fleshy, upper surface deeply rugose, the veins impressed, dark green, lighter underneath, glabrous, dull; young leaves and petioles covered with a light silky brown tomentum, with erose margins, the young leaves almost fringed; flowers numerous in subsessile clusters surrounding the stem, and hidden; calyx with prominent veins, curved, yellowish brown, five-cleft, bi-labiate, the lower lip consisting of two sepals, 12 mm. long, suddenly narrowing into filiform apices, the upper lip of three sepals half as long as lower lip, the two outer ones only beaked, all parts covered with a silky brown tomentum; corolla enclosed in the calyx, white, 36 mm. long including the 25 mm. long tube, slightly curved, two upper petals rounded and smaller than the three lower which are acute, pubescent; stamens adhering in the lower half of the tube, the filament 10 mm. long; style 14 mm. long, green, thickening towards the base; stigma flattened, two-lobed, the lobes obtuse, 2 mm. long; fruit ovoid (immature), the calyx deciduous from fruit, on pedicels of 8–10 mm., and covered in its young state with a brown tomentum.

KAUAI: Forests of Kaholuamano, elevation 4,000 ft., on cliffs, near streams or waterfalls, along the trail of the Waialae Valley, flowering March 3, 1909, Rock, no. 2282 in herb. College of Hawaii.

One of the most striking species of Cyrtandra. It resembles a species of Cyanea of the section Palmaeformes, hence the specific name. The native name of this species is *Mapele*.

CYRTANDRA FILIPES Hillebr. Fl. Haw. Isl. 336. 1888

"Habit of *C. grandiflora;* leaves three or four in a whorl, flaccid, pale underneath and glabrate, oblanceolate, 10–15 cm. long, 2.5–3.75 cm. wide, on petioles of 8–12 mm., acuminate at both ends, remotely dentate or serrulate; flower solitary on a short peduncle of 1–6 mm., the slender pedicels many times longer, 18–36 mm.; bracts filiform, 4–6 mm., soon caducous; calyx herbaceous, glabrous, cylindrical or campanulate, 12–24 mm., bilabiately five-fid to less than the middle, into sharply pointed, lanceolate lobes, deciduous from the fruit; corolla glabrous, as long as the calyx or longer, 20–28 mm., shaped as in *C. grandiflora;* ovary glabrous; berry slender fusiform 18 mm."[1]

WEST MAUI: Gulches of Honokawai and Kaanapali, Hillebr., without date or number in herb. Berlin, part of type in herb. College of Hawaii.

The writer is only acquainted with this species from Hillebrand-material in the Berlin herbarium. It is related to the writer's *C. longifolia* var. *calpidicarpa.*

CYRTANDRA GRANDIFLORA Gaud. Bot. Voy. Uranie 447. *pl. 55.* 1826

Cyrtandra Endlicheriana Walp. Nov. Act. Nat. Cur. 19, Suppl. 1. 359. *t. 10.* 1843.

Cyrtandra Ruckiana Meyen Reise 2, 125. 1834.

A small shrub 1–2 m. high; branches quadrangular, the new parts ferruginous-tomentose; leaves opposite, elliptical, acuminate at both ends, subentire, 10–14 cm. long, 4–8 cm. broad, obscurely crenulate, thin, chartaceous, glabrate above, pilose underneath especially along the midrib and nerves; petioles 2 cm.; cymes few-flowered; peduncle 2–6 cm., 1–7-flowered; bracts foliaceous, 2–3 cm., ovate-lanceolate, subpetiolate or clasping at the base, greenish, deciduous; pedicels 0–1 cm.; calyx herbaceous, campanulate, 18–24 mm. long, unequally five-fid, the lobes broadly triangular, acute; corolla large, glabrous, exserted, 30–32 mm., the tube curved, the limb bilabiate, large, spreading; ovary glabrous, style articulate at the base; fruit 16 mm. long, 8 mm. broad, ellipsoidal, glabrous, white, the calyx deciduous.

INSULIS SANDWICENSIBUS, GAUDICHAUD.

OAHU: Beechey in herb. Kew; Meyen, flowering specimen in herb. Berlin, two sheets, one labeled *Cyrtandra Rucriana*, only leaf-specimen, det. by C. B. Clarke as *C. grandiflora;* Mann et Brigham no. 40 in herb. Kew; Nuttall in herb. British Museum; Nuuanu

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[1] Hillebrand's description is here quoted, only the measurements have been changed from inches to the metric system.

Valley, flowering 1868, Hillebrand, without number, in herb. Berlin; Wawra no. 1746 (flowering), in herb. Vienna (four sheets); Pauoa Valley, flowering, Nov. 4, 1908, Rock no. 704; same locality, flowering Oct. 29, 1909, Rock no. 10346.

In the Berlin herbarium with the Hillebrand material, is a sheet labeled *C. grandiflora*, collected in the Malay peninsula, State of Pahang in 1909, no. 13673; this plant does not belong to our *C. grandiflora* Gaud.

The calyx and flowers of that specimen are silky tomentose, and in other respects it does not agree with our plant. The Meyen specimen is labeled *C. Rucriana*, while C. B. Clarke cites it in his monograph as *C. Ruckiana.*

A very distinct species common in the valleys back of Honolulu, a branching shrub with large white attractive flowers; occurs only at lower elevations of 500–1,000 feet, usually in dense shade along water courses.

CYRTANDRA OENOBARBA Mann, Proc. Amer. Acad. **7**: 189. 1866

Low, decumbent, 3–6 dm. high, fleshy, the stem and petioles shaggy with stiff, dark-brown, reflected hairs; leaves ovate or subcordate, acute at the apex, denticulate, hirsute with reddish hairs along the veins underneath, glabrate above, fleshy, 6.25–8.75 cm. long, about 6 cm. wide, on stout petioles of 2.5–5 cm.; peduncle one- to two-flowered, as long as the petioles; calyx shaggy, oblong, 5-fid, the lobes ovate lanceolate, sharply acuminate, foliaceous; corolla slightly exserted, fully 2.5 cm. long, glabrous, the limb large, spreading.

KAUAI: Wahiawa falls and Waioli, foot of Waialeale, Mann and Brigham no. 616.

It seems that this species has only been collected by Mann, for the writer found no material of it in any of the collections, neither in those of Wawra nor Hillebrand. C. B. Clarke in his monograph says also "species non visa."

The writer is not acquainted with the species but with the variety *petiolaris* Wawra.

CYRTANDRA OENOBARBA Mann var. PETIOLARIS Wawra, Flora **55**: 563. 1872

Cyrtandra oenobarba Mann var. *rotundifolia* Wawra, l. c.

Cyrtandra oenobarba Mann var. *obovata* Hillebr. Fl. Haw. Isl. 338. 1888.

Plant low, procumbent, 12–36 cm. high; leaves opposite, elliptical, acute or rounded at both ends, 10–14 cm. long, 6 cm. wide, remotely serrate, glabrate above, with blackish-brown tomentum along the midrib and veins, otherwise pale and glabrate; petioles 5–7 cm., with blackish hair; peduncle very short 5 mm., densely villous with blackish hair; bracts 8 mm., oblong, deciduous; pedicels 2–4, 0–8 mm. long; calyx 2 cm. long, narrow, tubular, divided to the middle into 5, linear-lanceolate lobes, covered with blackish to yellowish hair; corolla 3 cm., glabrate; fruit 16 mm. long, 3 mm. broad, narrow cylindrical, the calyx persistent.

KAUAI: Wawra no. 2012, 2157 in herb. Vienna, and portion of type of no. 2012 in herb. College of Hawaii; Hanapepe fall, Abbe Faurie, flowering Dec. 1909, no. 625 (distributed as *C. oenobarba* Mann), in the herb. College of Hawaii, as no. 12520.

A distinct variety, differing from the species in the long petioles, very short peduncle, and pedicels; it is identical with Wawra's var. *rotundifolia* which seems to differ from it only in the glabrous leaves.

CYRTANDRA OENOBARBA Wawra var. HERBACEA (Wawra) Heller, Minn. Bot. Stud. **9**: 890. 1897

Cyrtandra paludosa Gaud. var. *herbacea* Wawra, Flora **55**: 559. 1872.

Herbaceous, procumbent; branches fleshy, villous with reddish to grayish hair; leaves fleshy, elliptical or subovate, 17 cm. long, 6–10 cm. wide, coarsely serrate, on petioles of 2–6 cm.; peduncles very short, 0–9 mm., many-flowered; pedicels short, often 0–7 mm.; calyx glabrous; corolla large, curved, glabrous; fruit unknown.

KAUAI: Hanapepe falls, Wawra no. 2070 in herb. Vienna; same locality, July, Heller no. 2490 in part, distributed as *C. oenobarba.*

Wawra's specimen no. 2070 is a distinct variety but comes close to *C. oenobarba* var. *petiolaris.* It has nothing in common with *C. paludosa.*

COLLEGE OF HAWAII, HONOLULU

ON THE DISTRIBUTION OF ABNORMALITIES IN THE INFLORESCENCE OF SPIRAEA VANHOUTTEI

J. Arthur Harris

I. Introductory Remarks

Experimental breeders, primarily de Vries, have shown that in certain races individuals of more or less mutually exclusive characteristics may regularly occur in fairly constant proportions. Students of hybridization have devoted their chief effort for the past fifteen years to a study of the laws of segregation of parental characters in filial generations. Those interested in experimental morphology recognize the fact that a pure bred individual may in the course of its ontogeny display characteristics which might belong to distinct varieties or species.

In its relation to both genetic and morphogenetic problems the investigation of the distribution of abnormalities among the synchronously developed organs of the same individual seems of importance.

The purpose of the present note is to call attention to peculiarities of the frequency disrtibutions of certain abnormalities of the pedicels in one of the most splendid garden spiraeas, *S. Vanhouttei.*

II. History of Spiraea Vanhouttei and Discussion of Morphology of Inflorescence

1. *History of S. Vanhouttei* (Briot) Zbl.

Briot writes of the origin of the form which he refers to as *Spiraea aquilegifolia vanhouttei*:[1] "Cette variété, obtenue par M. Billard . . . de graines du *Spiraea aquilegifolia*." He also states: "Le *Spiraea aquilegifolia* est, dit-on, une forme du *Spiraea trilobata*." In his original description Zabel[2] gives no statement as to where or when this "hybrid" was formed. In a later paper[3] he merely refers to it as a hybrid between *Spiraea cantoniensis* and *S. trilobata*, without giving any details or actual proof of its hybrid origin.

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[1] Briot, Rev. Hort. **37**: 269. 1866.

[2] Zabel, H., Gart. Zeit. **3**: 496. 1884.

[3] Zabel, H., Mitteil. Deutsch. Dend. Ges. **1904**: 59.

Schneider[4] follows Zabel in regarding the form as a hybrid between *S. cantoniensis* and *S. trilobata.* So far as I can make out there is no really valid ground for this conclusion.

The early writers on *S. vanhouttei* noted abnormalities of the inflorescence. Briot[5] describes some in the original material. Zabel[6] has even described a new form, which he calls *S. Vanhouttei* var. *phyllothyrsa*, in part distinguished by abnormalities of the inflorescence.

Those who desire to compare the anomalies described in this paper with those hitherto recorded may consult these papers. My purpose has not been to describe in detail all the types of aberration which may occur, but rather to throw them into categories usable for statistical analysis.

2. *Descriptive Morphology of Inflorescence*

The normal inflorescence of *S. Vanhouttei* is a many-flowered umbel-like raceme. In general, the pedicels originate fairly close together, but occasionally the lowermost flowers are considerably scattered.

Normally each ray, as I shall sometimes call the pedicels, is simple, terminated by a single flower, but occasionally more or less compounded. The normal inflorescence, composed exclusively of simple rays, is too familiar to require illustration. Figs. 17 and 18 give a good idea of the abnormal inflorescence, the latter figure representing a rather advanced though by no means extreme stage of compounding.

In general it is the lowermost rays of the flower cluster which become compound, but there are inflorescences, and perhaps individual plants, in which this is not true.

The range of variation in the abnormal pedicels is, as shown in Figs. 1–16,[7] of the two plates, very great. In the earlier work with the form I devoted much attention to the attempt to classify the various anomalies into logical groups: for example, to distinguish between synanthies and the compounding of the flower stalk, and between synanthies and the production of an accessory pedicel immediately below the terminal receptacle.

[4] Schneider, C. K., Illust. Hand. Laubholzk. 465. 1905.

[5] Briot, Rev. Hort. **37**: 269. 1866.

[6] Zabel, H., Mitteil. Deutsch. Dend. Ges. **1904**; 59–60.

[7] Figs. 1–16 are natural size, Figs. 17–18 twice natural size.

Typical cases of synanthy are shown from the side in Figs. 5, 6, 8 and 9 and from below in Figs. 2 and 3, to which Fig. 1 of a normal flower is joined for comparison. Examples of the production of an accessory pedicel below the normal receptacle are shown from the side in Fig. 10 and from below in Fig. 4. But all possible gradations may be found between these two types of anomalies: hence it is idle to recount the criteria which have been applied in an attempt to distinguish between them. For example, it is difficult to decide just how the cases illustrated in Figs. 7, 11, 12 and 14 shall be classified. They combine in some degree the characteristics of perfectly constituted secondary inflorescences, of synanthous flowers and those in which there is a production of an adventitious pedicel from below the receptacle.

The numbers of flowers involved in synanthy varies considerably. Generally it is 2, but 3, 4 or 5 may be found. Figs. 2–3, 5–11, and 14 serve as illustrations. The number of secondary rays originating below the inflorescence is also variable. It is interesting to note that very frequently, and I believe in the great majority of the cases, the secondary pedicel extends considerably above the flower below which it originates, as shown in Figs. 10 and 12.

Between synanthy, or pedicels showing secondary rays inserted below the receptacle, to the most perfectly formed secondary "umbels," as shown in Fig. 16, all possible transitions, both in number of flowers and perfection of formation, are found.

Ordinarily the rays of the secondary umbels are inserted at about the same position, but occasionally examples are found in which one ray is considerably lower than the rest, or in which the lowermost rays are rather scattered. The number of secondary pedicels varies greatly.

Some abnormalities of inflorescence structures are almost invariably formed in any large series of plants. Without going into details concerning the general observations of the past several years, I think it may be safely stated that variation in the inflorescence is to some extent dependent upon the peculiarities of the individual plants and to some extent determined by environmental conditions.

III. Discussion and Analysis of Data

The first problem to require consideration is that of the frequency and the nature of the distribution of abnormal pedicels.

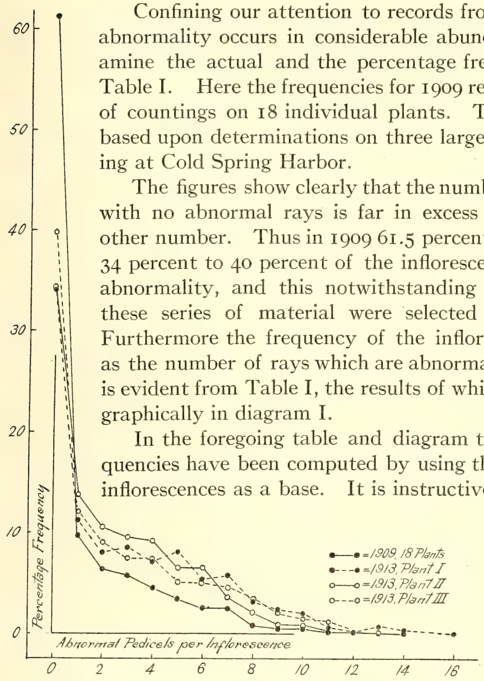

DIAGRAM 1. Percentage frequencies of number of abnormal pedicels per inflorescence in all inflorescences.

Confining our attention to records from plants in which abnormality occurs in considerable abundance, we may examine the actual and the percentage frequencies given in Table I. Here the frequencies for 1909 represent the results of countings on 18 individual plants. Those for 1913 are based upon determinations on three large individuals growing at Cold Spring Harbor.

The figures show clearly that the number of inflorescence with no abnormal rays is far in excess of these with any other number. Thus in 1909 61.5 percent and in 1913 from 34 percent to 40 percent of the inflorescences were without abnormality, and this notwithstanding the fact that all these series of material were selected for abnormality. Furthermore the frequency of the inflorescences decreases as the number of rays which are abnormal increases. This is evident from Table I, the results of which are represented graphically in diagram I.

In the foregoing table and diagram the percentage frequencies have been computed by using the total number of inflorescences as a base. It is instructive to determine the relative frequencies of different number of abnormalities in the inflorescences which have at least one abnormal pedicel. The results are shown in Table II, and represented graphically in diagram 2.

Both methods give skew distribution, the highest frequency falling on 0 or 1 abnormal ray, and the frequency decreasing from this class to those with higher numbers of abnormal pedicels.

That the skewness of distribution of the number of abnormal pedicels per inflorescence is not due to skewness in the distribution of number of pedicels in the inflorescence as a whole is shown clearly by diagram 4, which gives the percentage frequencies of number of pedicels in three of the series. All of these distributions are fairly symmetrical.

TABLE I

Actual Frequencies, f, and Percentage Frequencies of Number of Abnormal Pedicels per Inflorescence in All the Inflorescences

Number of Abnormal Pedicels	1909, 18 Plants		1913, Plant I		1913, Plant II		1913, Plant III	
	f	%	*f*	%	*f*	%	*f*	%
0	1,255	61.51	388	34.18	334	34.25	364	39.91
1	203	9.95	130	11.45	136	13.95	111	12.17
2	132	6.47	93	8.19	105	10.77	82	8.99
3	120	5.88	98	8.63	94	9.64	68	7.46
4	95	4.66	83	7.31	89	9.13	66	7.24
5	71	3.48	95	8.37	65	6.67	46	5.04
6	55	2.69	64	5.63	65	6.67	48	5.26
7	54	2.65	68	5.99	37	3.79	42	4.61
8	19	.93	38	3.35	22	2.26	30	3.29
9	11	.53	29	2.56	10	1.02	21	2.30
10	12	.59	23	2.03	8	.82	16	1.75
11	7	.34	9	.79	3	.31	13	1.43
12	5	.24	3	.26	3	.31	3	.33
13	—	—	9	.79	2	.21	1	.11
14	1	.04	4	.35	2	.21	1	.11
15	—	—	—	—	—	—	—	—
16	—	—	1	.08	—	—	—	—
Total......	2,040	99.96	1,135	99.96	975	100.01	912	100.00

TABLE II

Percentage Frequencies of Number of Abnormal Pedicels in Abnormal Inflorescences Only

Number of Abnormal Pedicels	Percentage Frequency			
	1909, 18 Plants	1913, Plant I	1913, Plant II	1913, Plant III
1	25.86	17.40	21.22	20.26
2	16.82	12.44	16.38	14.96
3	15.28	13.12	14.66	12.41
4	12.10	11.11	13.88	12.04
5	9.04	12.72	10.14	8.40
6	7.01	8.56	10.14	8.76
7	6.87	9.10	5.77	7.66
8	2.42	5.08	3.43	5.47
9	1.40	3.88	1.56	3.83
10	1.52	3.08	1.25	2.92
11	.89	1.20	.47	2.37
12	.63	.40	.47	.55
13	—	1.20	.31	.18
14	.12	.54	.31	.18
15	—	—	—	—
16	—	.13	—	—

The fact that the three collections made from large individuals in the spring of 1913 show the same type of frequency distribution as the massed materials of 1909 is sufficient proof that the skew distribution of number of abnormal rays per inflorescence is not due to any

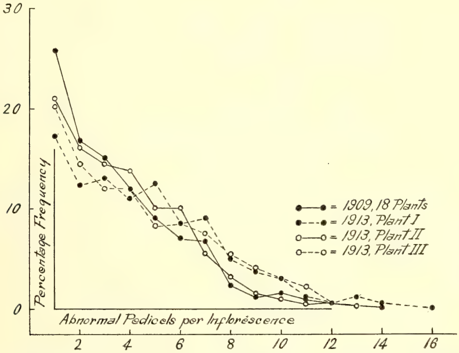

DIAGRAM 2. Percentage frequencies of number of abnormal pedicels per inflorescence in abnormal inflorescences.

TABLE III

Number of Abnormal Pedicels per Inflorescence in Inflorescences of Various Total Numbers of Pedicels

Total Pedicels in Inflorescence	Abnormal Pedicels													Total
	1	2	3	4	5	6	7	8	9	10	11	12	14	
3–5...	—	—	1	—	—	—	—	—	—	—	—	—	—	1
6–8...	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9–11...	1	1	—	—	—	—	—	—	—	—	—	—	—	2
12–14...	11	3	—	—	—	—	1	—	—	—	—	—	—	15
15–17...	23	13	12	8	10	2	3	—	—	—	—	—	—	71
18–20...	54	43	33	31	16	11	12	—	1	3	1	1	—	206
21–23...	70	38	37	31	24	17	19	8	5	3	4	2	—	258
24–26...	27	20	23	18	12	19	16	9	5	4	2	1	1	157
27–29...	14	7	9	4	7	5	3	2	—	2	—	1	—	54
30–32...	3	5	4	3	2	1	—	—	—	—	—	—	—	18
33–35...	—	2	—	—	—	—	—	—	—	—	—	—	—	2
36–38...	—	—	1	—	—	—	—	—	—	—	—	—	—	1
Total..	203	132	120	95	71	55	54	19	11	12	7	5	1	785

process of combination of materials from individuals differentiated with respect of the number of abnormal rays which they produce.

Table III shows the distribution of number of abnormal rays per inflorescence in groups of inflorescences of similar numbers of total rays in the data for 1909. In this table are included wholly normal inflorescences, all of which are entered in the zero class, as well as the inflorescences which contain abnormalities. Whenever the countings are sufficiently numerous to justify conclusions, the frequency distri-

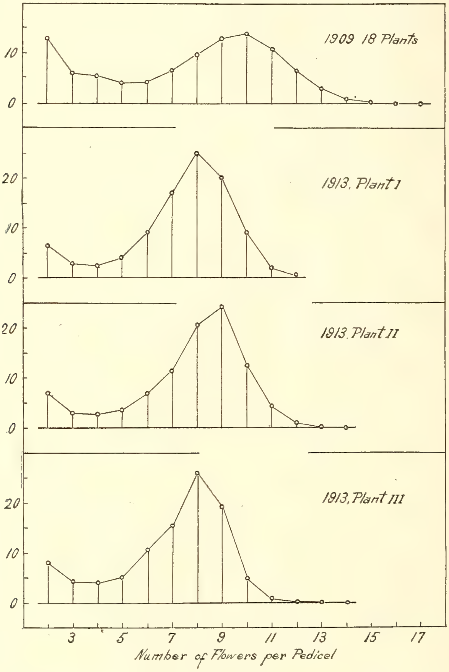

DIAGRAM 3. Percentage frequencies of number of abnormal flowers per pedicel in abnormal pedicels. Note the bimodal nature of the distribution.

bution of numbers of abnormal pedicels per inflorescence in groups of inflorescence with similar number of pedicels is skew, just as it is in the series as a whole.

The second peculiarity of the distribution of abnormality in the inflorescences of this species is to be seen in the frequency of numbers of flowers per pedicel. This is excellently shown for a combined series of countings made from 18 shrubs in 1909 and from three large individual plants examined in 1913. These frequencies, reduced to a percentage basis, are represented graphically in diagram 3.[8]

Table IV

Bimodal Distribution of Number of Flowers per Pedicel in the Abnormal Inflorescences of Individual Plants

Plant Number	Number of Flowers Produced by Abnormal Pedicels																Total
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
1	27	5	7	9	11	15	31	31	59	40	17	8	1	–	–	–	261
2	1	3	–	2	1	3	5	3	2	–	–	–	–	–	–	–	20
3	11	6	9	6	8	19	8	2	–	–	–	–	–	–	–	–	69
4	26	15	11	6	8	6	6	16	9	6	2	–	–	–	–	–	111
5	8	4	8	5	4	10	12	24	13	7	3	–	–	–	–	–	98
6	22	7	10	4	6	12	10	16	19	12	6	1	–	–	–	–	125
7	12	9	7	10	12	7	15	13	8	3	–	–	–	–	–	–	96
8	31	25	15	11	7	14	12	31	36	53	62	55	27	9	1	–	389
9	20	6	14	7	9	10	8	15	10	8	3	–	–	–	–	–	110
10	8	3	2	1	1	4	6	6	4	–	–	–	–	–	–	–	35
11	21	11	5	5	3	4	21	22	15	9	3	–	–	–	–	–	119
12	3	3	3	–	4	13	8	3	–	–	–	–	–	–	–	–	37
13	41	31	16	14	16	13	31	50	47	28	21	4	1	–	–	–	313
14	19	10	9	7	3	8	10	12	18	26	11	3	–	–	–	–	136
15	34	10	7	10	9	9	22	35	42	41	26	6	2	–	–	–	253
16	23	12	11	9	8	25	32	33	21	7	4	–	–	–	–	–	185
17	22	7	6	6	6	8	11	14	15	6	6	3	1	–	–	–	111
18	35	6	9	9	8	8	26	47	81	61	28	5	–	–	–	1	324
Total..	364	173	149	121	124	188	274	373	399	307	192	85	32	9	1	1	2,792

In all four series there is a major mode on 8, 9 or 10 flowers per pedicel and a secondary mode on two flowers per pedicel.

This species adds, therefore, one other to the series of dimorphic characters, which have been reviewed elsewhere.[9]

That the bimodal condition for the 1909 series is not due to the mixing of inflorescence from a series of shrubs, some of which have a modal frequency of two flowers per pedicel in the abnormal pedicels and other shrubs which have a modal frequency on a higher number of flowers in the secondary umbels, as we may for convenience call them, is shown at once by an examination of the frequency distributions for number of flowers per pedicel in the abnormal pedicels of the individual shrubs. This is given in Table IV. From these data it appears that 16 of the 18 individual plants which contributed to the total series of 2,792 abnormal rays upon which the polygon for 1909 in diagram 4 is based, show a distinct secondary mode on 2 flowers per pedicel.

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[8] A few synanthous flowers have been included among the two-flowered pedicels.

[9] Harris, J. Arthur, A Bimodal Variation Polygon in *Syndesmon thalectroides* and its Morphological Significance, Amer. Nat. 44: 19–30. 1910.

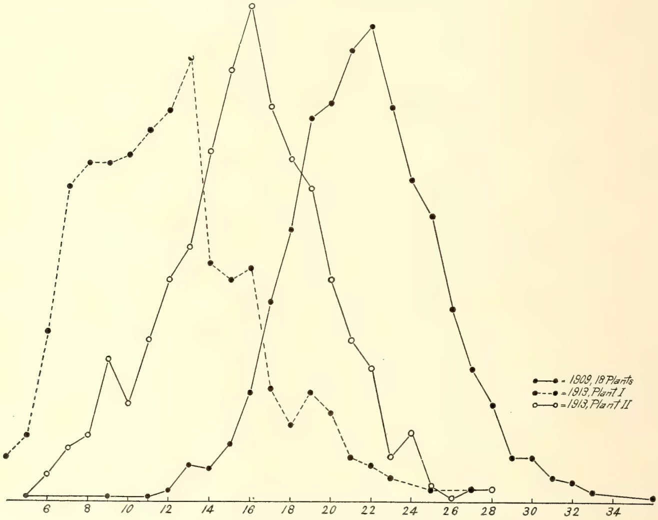

Diagram 4. Frequencies of number of pedicels in *Spiraea*.

The two plants which are exceptions to this rule are represented by only 20 and 37 pedicels each. These numbers are entirely too small to justify any conclusion concerning the nature of the distributions. Had larger series of countings from these individuals been available, they might have shown the same bimodal condition as the other sixteen.

The whole series of available data may be taken to indicate with remarkable consistency that the bimodal nature of the distribution of number of flowers per pedicel is not due to heterogeneity of material, so far as this may originate from the combination of inflorescences derived from differentiated individuals. It represents, therefore, the resultant of some group of factors innate in the individual.

Having eliminated the possibility of an influence of the individual plant as a determining factor of the peculiar frequency distribution, it seems worth while to enquire whether any characteristic of the inflorescence itself may have an influence upon the distribution of the number of flowers per pedicel. It is quite conceivable, for example, that the inflorescences which are the most highly abnormal as measured by the total number of abnormal pedicels, should have a larger number of flowers in their secondary umbels than those which are only very slightly abnormal. The combination of a series of inflorescences, some only slightly abnormal and others highly abnormal, might, under such conditions, result in a bimodal distribution of flower number in the series of pedicels from the combined inflorescences.

TABLE V

Bimodal Distribution of Number of Flowers per Pedicel in Inflorescences with Various Numbers of Abnormal Flowers per Pedicel

Total Abnormal Pedicels	Number of Flowers Produced by Abnormal Pedicels											Total
	2	3	4	5	6	7	8	9	10	11	12	
1	30	8	5	8	12	18	28	12	6	3	–	130
2	17	13	9	8	19	33	31	35	17	4	–	186
3	27	13	13	12	32	52	63	50	28	4	–	294
4	21	7	20	18	42	58	62	62	33	9	–	332
5	34	15	13	19	40	66	131	103	41	12	1	475
6	24	16	4	15	31	56	89	91	47	10	1	384
7	26	6	9	23	49	79	132	96	44	12	–	476
8	15	4	4	11	20	50	80	64	42	13	1	304
9	16	7	7	7	29	54	59	59	20	3	–	261
10	8	2	1	11	20	54	67	46	16	5	–	230
11	2	3	1	2	12	16	29	28	6	–	–	99
12	1	–	1	3	1	4	13	10	2	1	–	36
13	2	–	–	–	7	20	42	26	18	2	–	117
14	2	–	–	1	4	15	20	12	1	1	–	56
16	–	–	–	1	1	5	5	4	–	–	–	16
Total......	225	94	87	139	319	580	851	698	321	79	3	3,396

Table V shows the number of flowers per pedicel in the 3,396 abnormal pedicels produced by the 747 inflorescences examined from

Plant I in 1913, arranged according to the number of abnormal pedicels per inflorescence. This table is quite typical of the others which have been made. The bimodal distribution characterizes all the arrays in which the number of observations is large enough to give the distributions critical value.

Differentiation between individual plants and differentiation due to correlation between the characteristics of the pedicels and those of the inflorescence would seem to be the most probable source of a spurious bimodality in the frequency distributions of number of flowers per pedicel. That neither of these factors underlies the observed form of the frequency distribution seems quite clear from the foregoing tables. Bimodiality seems rather to be due to innate factors operative in the morphogenesis of the individual pedicels.

Now in examining the graphs on diagram 3 the reader will note that the mode on 2 flowers per pedicel is but a transition stage to the higher mode—not represented in the diagram—on a single flower per pedicel—that is to the normal condition.

TABLE VI

Distribution of Number of Flowers per Pedicel for All Pedicels

Number of Flowers per Pedicel	Series			
	1909, 18 Shrubs	1913, Plant I	1913, Plant II	1913, Plant III
1	14,250	8,436	5,196	5,714
2	364	225	177	193
3	173	94	83	103
4	149	87	72	101
5	121	139	87	119
6	124	319	159	245
7	188	580	280	367
8	274	851	506	616
9	373	698	601	458
10	399	321	305	114
11	307	79	112	22
12	192	3	35	8
13	85	—	9	3
14	32	—	1	1
15	9	—	—	—
16	1	—	—	—
17	1	—	—	—
Total........	17,042	11,832	7,623	8,064

Tabulating the actual number of flowers per pedicel for all pedicels examined, the frequencies in Table VI are obtained.

These seriations bring out clearly that there are minimum frequencies on four flowers per pedicel, and that the number of pedicels increases rapidly as the number of flowers becomes smaller, reaching a maximum on the single flowered (normal) pedicel, and that on the other hand it increases more gradually as the number of flowers becomes larger to a secondary maximum on 8–10 flowers per pedicel.

Concluding Remarks

This paper provides illustrations of the chief types of variation which occur in the inflorescence of *Spiraea Vanhouttei*, and records the results of statistical studies of the distribution of these abnormalities.

The inflorescence of *S. Vanhouttei* is described by taxonomists as a simple raceme-like umbel. A number of the pedicels are, however, often compound. The compounding may range from a simple synanthous condition to the production of a perfect, many-flowered, secondary umbel in the place of the solitary flower which normally terminates the pedicel.

The distribution of abnormal pedicels among the inflorescences in plants in which abnormality is of frequent occurrence is not to be represented by a normal or Quetelet's curve but forms a one-sided or skew frequency distribution, in which the frequency of occurrence decreases as the number of abnormal pedicels per inflorescence becomes larger. This is the first law of variation in the abnormalities of the inflorescence.

If the normal pedicels be included with the abnormal to form a single frequency distribution of number of flowers per pedicel in inflorescences in which some of the pedicels are abnormal, it will be seen that the two flowered pedicels form a transition frequency from classes of larger numbers of flowers to those with a single flower per inflorescence.

On examining these bimodal frequency distributions the reader may be tempted to formulate some hypotheses concerning the coexistence of determiners of mutually exclusive characters or concerning the segregation of alternative, but variable, characters in the morphogenetic processes of the individual plants. *S. Vanhouttei* is known to be of garden origin and is supposedly a hybrid between *S. cantoniensis* and *S. trilobata*. The evidences are not, however, such as would be readily accepted by a careful geneticist. Furthermore, both of these assumed parent species have simple inflorescences.

S. Vanhouttei is horticulturally such a splendid form that it would be well worth while for some unoccupied geneticist to attempt further crosses in this group of Spiraeas.

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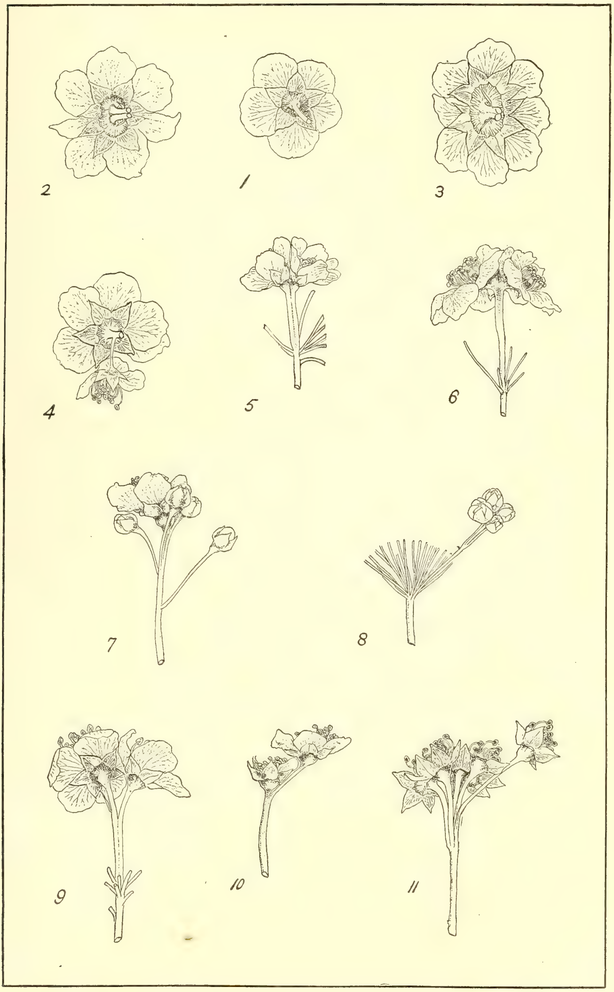
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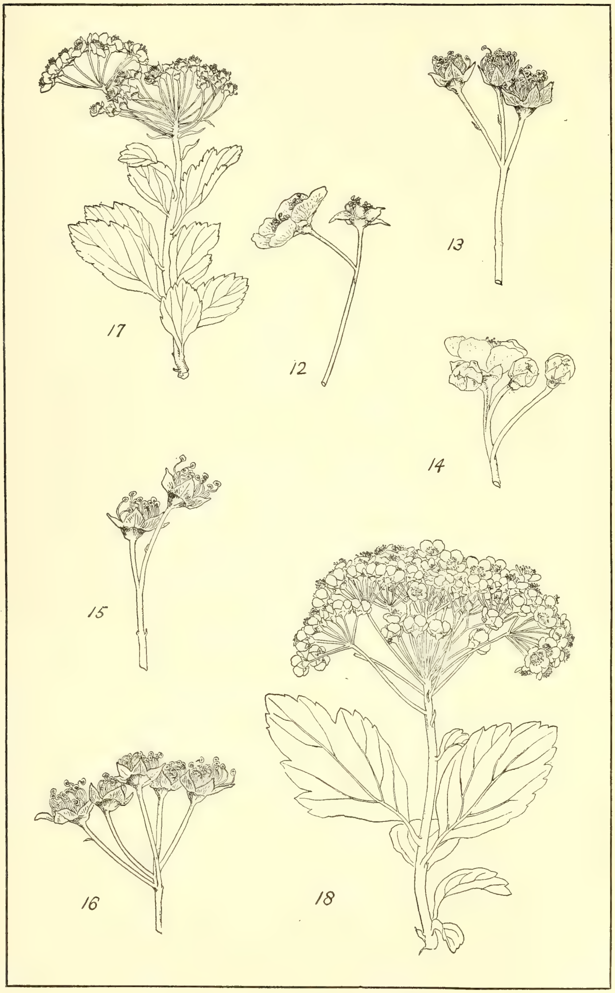
17
12
13
14
15
16
18

HARRIS: ABNORMALITIES IN SPIRAEA.

INDEX TO VOLUME IV

(New names and final members of new combinations are in **heavy-face** type.)

Abies grandis, duration of leaves in, 149; host of Uredinopsis, 332; *lasiocarpa*, host of Uredinopsis, 332
Abnormalities in the inflorescence of *Spiraea Vanhouttei*, 624
Achlya, a form lacking sexual reproduction, 354
Aecidium abscedens, 46; *Borreriae*, 46; *circumscriptum* (syn.), 49; *Cissi* (syn.), 49; *Clibadii* (syn.), 49; *decoloratum* (syn.), 49; *expansum* (syn.), 51; *passifloriicola*, 46; *Tournejortiae*, 46; *tubulosum*, 46
Agaricaceae, taxonomy of, 315
Agarics, development of, 221
Alternaria, taxonomic characters of, 439
Antagonistic action of other sugars toward galactose and mannose, 430
Arbutus menziesii, 153
Arctostaphylos tomentosa, *uva-ursa*, 154
Ascospore production, in Endothia, 18
Asthma, caused by wheat protein, 417

BARTLETT, H. H. (see La Rue, Carl D., 119)
Berberis aquifolia, *nervosa*, 154
BLIZZARD, A. W. The development of some species of Agarics, 221
Botryorhiza Hippocrateae, 47; 52

Carbohydrates, effect of, on development of rusts, 197
Carbon dioxide, effect of lack of, on development of rusts, 195
Castanea dentata, host of Endothia, 3, 112; *pumila*, host of Endothia, 3
Ceanothus velutinus, 153
Chestnut blight, 1
Chimaphila menziesii, *umbellata*, 154
Chlorophyll formation, influence of, 407
Cichorium intybus, fertility in, 375
Cissus sicyoides, host of Endophyllum, 49
Clibadium arboreum, *Donnell-Smithii*, *erosum*, *surinamense*, hosts of *Endophyllum decoloratum*, 50
Climatic factors, influence of, on the development of *Endothia parasitica*, 1
Clitocybe adirondackensis, *cerussata*, development of, 226, 228
Clitopilus noveboracensis, development of, 230
Coleosporium solidaginis, 334
Conductivity of electrolytes, 411
Cortinarius pholideus, development of, 520
Crown-rot of fruit trees: histological studies, 477
Cyrtandra, revision of the Hawaiian species of, 604
Cyrtandra cyaneoides, 620; *filipes*, 621; *grandiflora*, 621; *longifolia*, 611, var. *arborescens*, 611, var. **calpidicarpa**, 618, var. *degenerans*, 613, formae **auriculaefolia**, 618, **cymosa**, 616, **oppositifolia**, 616, **subglabra**, 615; *oenobarba*, 622, var. *petiolaris*, 622, var. *herbacea*, 623; *paludosa* var. *alnifolia*, 607, var. *brevicalyx*, 607, var. **Gayana**, 608, var. **haupuensis**, 609, var. *microcarpa*, 607, var. *subherbacea*, 608, var. *typica*, 605; **Waianuensis**, 618

Development of *Cortinarius pholideus*, 520
Development of some species of Agarics, 221
Drabas of North America, 253
Draba alpina, 257; *argyrea*, 266; **asterophora**, 263; *Bellii*, 261; **cruciata**, 265; **cyclomorpha**, 263; *Douglasii*, 259; *fladnizensis*, 257; **globosa**, 257; **incerta**, 261; **laevicapsula**, 262; *Lemmoni*, 263;

lonchocarpa, 262; **Mulfordae,** 264; **Nelsonii,** 259; *nivalis,* 262; *oligosperma,* 259; **oreibata,** 257; **pterosperma,** 266; **sphaerocarpa,** 266; **sphaeroides,** 265; **sphaerula,** 258; *subsessilis,* 267; *uncinalis,* 265; *ventosa,* 264; **vestita,** 261
Duration of leaves in evergreens, 145

Effect of soil temperature on the growth of bean plants and upon their susceptibility to a root parasite, 513
Electrolytes, measurement of the conductivity of, 411
Elliott, John A. Taxonomic characters of the genera Alternaria and Macrosporium, 439
Endemic flora, origin of, in Hawaii, 602
Endophyllum **circumscriptum,** 49, 52; **decoloratum,** 49, 52; **Stachytarphetae,** 50, 52; **Wedeliae,** 49, 52
Endophyllum-like rusts of Porto Rico, 44
Endosperm color, inheritance of, in maize, 396
Endothia parasitica, influence of climatic factors on the development of, 7; *gyrosa,* 7; *radicalis,* 7; Influence of temperature on growth of, 112
Endothia pigments, 336
Environment, as related to leaf-structure, 533
Evaporation, relation between, and plant succession, 161
Evergreens, duration of leaves in, 145

Fertility in *Cichorium intybus:* the sporadic occurrence of self-fertile plants among the progeny of self-sterile plants, 375
Fritillaria, rusts on, 368
Fusarium Martii phaseoli, root parasite of beans, 513

Galactose, toxicity of, for green plants, 430
Galls, produced by Gymnosporangia, on cedar, 241
Galvanometer, use of, with a 60-cycle alternating current in the measurement of the conductivity of electrolytes, 411
Gates, Frank C. The relation between evaporation and plant succession in a given area, 161
Gaultheria shallon, 154
Green, Newton B. The use of the vibration galvanometer with a 60-cycle alternating current in the measurement of the conductivity of electrolytes, 411
Grossenbacher, J. G. Crown-rot of fruit trees: histological studies, 477
Gunnera petaloidea Gaud., a remarkable plant of the Hawaiian Islands, 33; *manicata,* 33
Gymnosporangium globosum, producing galls on cedar, 246; *Juniperi-virginianae,* producing galls on cedar, 241

Halorrhagaceae, 35
Hanson, Herbert C. Leaf-structure as related to environment, 533
Harris, J. Arthur. On the distribution of abnormalities in the inflorescence of *Spiraea Vanhouttei,* 624; and Lawrence, John V. The osmotic concentration of the tissue fluids of Jamaican montane rain-forest vegetation, 268
Harvey, R. B. and True, R. H. The influence of light and chlorophyll formation on the minimum toxic concentration of magnesium nitrate for the squash, 407
Hawaii, *Gunnera petaloidea* occurring in, 33; phytogeography of Manoa Valley in, 561
Hawaiian species of the genus Cyrtandra, section Cylindrocalyces Hillebr., 604
Hawkins, Lon A. and Stevens, Neil E. Endothia pigments I, 336
Hippocratea volubilis, host of Botryorhiza, 48
Hubert, Ernest E. (See Weir, James R., 327)

Humidity, effect of, on development of rusts, 188

Immunochemical studies of the plant proteins: proteins of the wheat seed and other cereals, 417
Influence of certain climatic factors on the development of *Endothia parasitica* (Murr.) And., 7
Influence of temperature on the growth of *Endothia parasitica*, 112
Inheritance of endosperm color in maize, 396

Jamaican montane rain-forest vegetation, 268
Juniperus scopulorum, duration of leaves in, 149

Kalmia polifolia, 154
KNUDSON, LEWIS. The toxicity of galactose and mannose for green plants and the antagonistic action of other sugars toward these, 430

LA RUE, CARL D. and BARTLETT, H. H. Matroclinic inheritance in mutation crosses of *Oenothera Reynoldsii*, 119
LAWRENCE, JOHN V. (See Harris, J. Arthur, 268)
Leaf-structure as related to environment, 533
Ledum groenlandicum, 154
Light and chlorophyll formation, influence of, on the minimum toxic concentration of magnesium nitrate for the squash, 407
Light, effect of, on development of rusts, 191
Linnaea americana, 153
LUTZ, ANNE M. Fifteen- and sixteen-chromosome Oenothera mutants, 53

MACCAUGHEY, VAUGHAN. *Gunnera petaloidea* Gaud., a remarkable plant of the Hawaiian Islands, 33; The phytogeography of Manoa Valley, Hawaiian Islands, 561
Macrosporium, taxonomic characters of, 439
Magnesium nitrate, toxic concentration of, 407
MAINS, E. B. The relation of some rusts to the physiology of their hosts, 179
Maize, inheritance of endosperm color in, 396
Mannose, toxicity of, for green plants, 430
Manoa Valley, Hawaii, phytogeography of, 561
Matroclinic inheritance in mutation crosses of *Oenothera Reynoldsii*, 119
Micromeria douglasii, 153
Mikania cordifolia, *odoratissima*, hosts of Endophylloides, 51
Mineral salts, effect of, on development of rusts, 190
MURRILL, WILLIAM A. The taxonomy of the Agaricaceae, 315
Mutation, from *Xanthium canadense*, 43

Observations on forest tree rusts, 327; on an Achyla lacking sexual reproduction, 354
Oenothera albida, 72; *bipartita*, 63, 64; *gigas* × *Lamarckiana*, 68; *Lamarckiana*, 65; *Lamarckiana* × *gigas*, 67; *lata*, 55, 59; *lata* × *gigas*, 66
Oenothera Reynoldsii, matroclinic inheritance in mutation crosses of, 119; pedigree of, 122, 123
Oenothera mutants, with fifteen and sixteen chromosomes, 53
OLIVE, E. W. and WHETZEL, H. H. Endophyllum-like rusts of Porto Rico, 44
Omphalia chrysophylla, development of, 222
Opuntia megacantha, 572
Origin and development of the galls produced by two cedar rust fungi, 241
Osmotic concentration of the tissue fluids of Jamaican montane rain-forest vegetation, 268
Overwintering of rusts, 332

Oxycoccus oxycoccus intermedius, 155

Pachistima myrsinites, 155

PAYSON, EDWIN BLAKE. The perennial scapose Drabas of North America, 253

PEASE, VINNIE A. Duration of leaves in evergreens, 145

Perennial scapose Drabas of North America, 253

Phytogeography of Manoa Valley, Hawaiian Islands, 561

Picea sitchensis, duration of leaves in, 149

Pigments of Endothia, 336

Pinus contorta, monticola, duration of leaves in, 149

Proteins, immunochemical studies of wheat, 417

Pseudotsuga taxifolia, duration of leaves in, 150

Pteridium aquilinum pubescens, host of Uredinopsis, 331

Puccinia Scleriae, 46; *substriata*, 46; *coronata* and *Sorghi*, used in experiments on relation to physiology of their hosts, 185

Pucciniastrum pustulatum, 334

Raphanus sativus, viability of seeds of, as affected by high temperatures and water content, 229

REDDICK, DONALD. Effect of soil temperature on the growth of bean plants and upon their susceptibility to a root parasite, 513

REES, CHARLES C. The rusts occurring on the genus Fritillaria, 368

Relation between evaporation and plant succession in a given area, 161

Revision of the Hawaiian species of the genus Cyrtandra, 604

Rhamnus purshiana, duration of leaves in, 152

Rhododendron californicum, 155

ROCK, JOSEPH F. Revision of the Hawaiian species of the genus Cyrtandra, section Cylindrocalyces Hillebr., 604

Rubus laciniatus, pedatus, ursinus, 153, 154

Rusts, of Porto Rico, 44; relation of, to the physiology of their hosts, 179; of forest trees, 327; on cedar, producing galls, 241; occurring on the genus *Fritillaria*, 368

SAWYER, W. H., JR. The development of *Cortinarius pholideus*, 520

Self-fertile plants of Cichorium, 375

SHULL, CHARLES A. An interesting modification in Xanthium, 40

Spiraea Vanhouttei, distribution of abnormalities in, 624

Squash, used in light and chlorophyll formation experiments, 407

Stachytarpheta cayennensis, dichotoma, hosts of *Endophyllum Stachytarphetae*, 50

Sterility of seeds of *Oenothera Reynoldsii*, 125

STEVENS, NEIL E. The influence of certain climatic factors on the development of *Endothia parasitica* (Murr.) And., 1; The influence of temperature on the growth of *Endothia parasitica*, 112; (see Hawkins, Lon A., 336)

STOUT, A. B. Fertility in *Cichorium intybus:* the sporadic occurrence of self-fertile plants among the progeny of self-sterile plants, 375

Succession, relation between, and evaporation, 161

Taxonomy of the Agaricaceae, 315

Taxus brevifolia, duration of leaves in, 150

Temperature, effect of, on development of rusts, 187; affecting viability of seeds of radish, 229; effect of, on susceptibility of bean plants to a root parasite, 513; effect of, on growth of bean plants, 513

Thuja plicata, duration of leaves in, 150

Toxicity of galactose and mannose for green plants and the antagonistic

action of other sugars toward these, 430
Transpiration of Fraxinus, 533
Treviso, a red-leaved variety of chicory, 376
TRUE, R. H. (See Harvey, R. B., 327)
Tsuga heterophylla, duration of leaves in, 151

Uredinopsis pteridis, on *Abies grandis*, 329; on *Pteridium aquilinum pubescens*, 331
Uredo Trichiliae, 46
Uromyces **aecidiiformis**, 369; *Fritillariae*, 372; *Holwayi*, 369; *Miurae*, 371; *proëminens*, 46

Vaccinium ovatum, 155; *parvifolium*, 152
Valerianodes cayennensis (syn.), 50
Viability of radish seeds as affected by high temperatures and water content, 229

WAGGONER, H. D. The viability of radish seeds (*Raphanus sativus* L.) as affected by high temperatures and water content, 299
Wedelia trilobata, host of *Endophyllum Wedeliae*, 49
WEIMER, J. L. The origin and development of the galls produced by two cedar fungi, 241
WEIR, JAMES R. and HUBERT, ERNEST E. Observations on forest tree rusts, 327
WESTON, WM. H. Observations on an Achlya lacking sexual reproduction, 354
Wheat protein, immunochemical studies of, 417
WHETZEL, H. H. (See Olive, E. W., 44)
WHITE, ORLAND E. Inheritance of endosperm color in maize, 396
WODEHOUSE, R. P. Immunochemical studies of the plant proteins: proteins of the wheat seed and other cereals. Study IX, 417

Xanthium, an interesting modification in, 40
Xanthium canadense var. *globuliforme*, 41

Zea Caragua × California Golden Pop, inheritance of endosperm color in, 396

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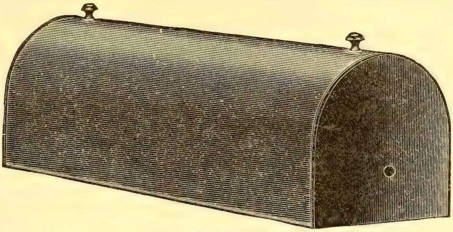

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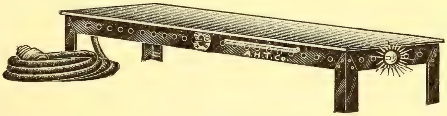

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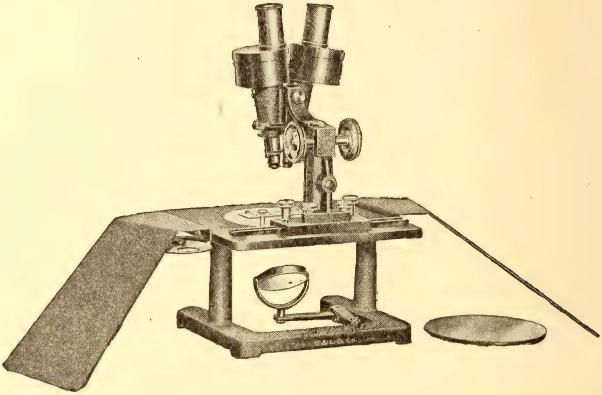

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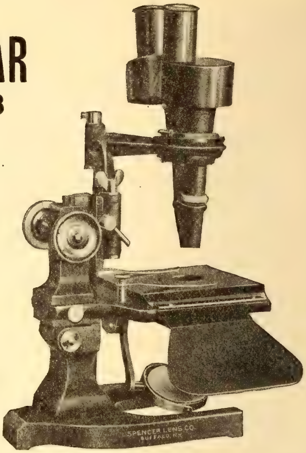

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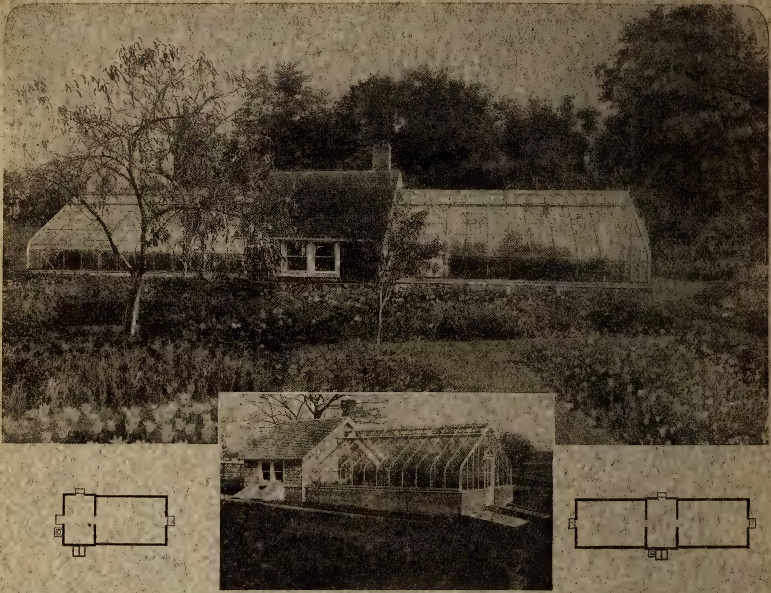

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